REVIEW

Insights into the Pathogenesis and Treatment of Cancer from Inborn Errors of Metabolism

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Mutations in genes that play fundamental roles in metabolic pathways have been found to also play a role in tumor development and susceptibility to cancer. At the same time, significant progress has been made in the treatment of patients with inborn errors of metabolism (IEM),[1] resulting in increased longevity and the unmasking of cancer predisposition, frequently hepatocellular carcinoma, in these conditions. These patients offer a potential opportunity to deepen our understanding of how intermediary metabolism impacts tumorigenesis. We provide an overview from the perspective of cancers in patients affected with IEM and discuss how dysregulation of these specific metabolic pathways might contribute to the mechanisms of cancer development and treatment.

Introduction

More than 50 years ago, Warburg initiated research on mitochondrial alterations in cancer and proposed a mechanism to explain the differences in energy metabolism between normal and cancer cells, suggesting that mitochondrial alterations could provide unique therapeutic targets in various cancer types.[2] Warburg's major insight was the proposition that metabolic changes observed in cancer are the primary changes that lead to its development rather than a secondary result of the malignant transformation.[2] Since then, several metabolic pathways have been found to be dysregulated in cancer, enabling cancer cells to acquire and metabolize nutrients necessary to meet the requirements of heightened proliferation. Understanding the existing cross-talk between cellular metabolism and growth control has resulted in a better understanding of normal and disease processes and facilitated the discovery of new treatment modalities in oncology, for example, trastuzumab and imatinib.[3,4]

Inborn errors of metabolism (IEM)[1] result in the disruption of a wide range of metabolic pathways including but not limited to the metabolism of proteins, carbohydrates, lipids, nucleic acids, steroids, and metals. IEM can result from a deficiency or overactivity of an enzyme, a deficiency of a cofactor required for enzymatic activity, an abnormality in degradation or in the transport processes that leads to the accumulation of upstream metabolites, a deficiency of a downstream metabolite, or a diversion of the affected metabolic flux to secondary pathways.[5] The availability of lifesaving treatments for patients with IEM has made early diagnosis of metabolic disorders crucial, thus stimulating the adoption of mandatory neonatal screening programs resulting in at least a 5-fold increase in the annual detection rate as compared to the previous 20 years.[6] This increase is at least partially explained by the diagnosis of infants with milder forms of IEM who would not have come to medical attention without the introduction of expanded newborn screening. Several large screening programs, such as the New England Newborn Screening Program and Pediatrix Analytical, have estimated that 1 in 4000 newborns might have a confirmed metabolic disorder.[7,8] As a result, prompt diagnosis with early implementation of tailored therapy has resulted in more successful interventions and, most importantly, improved survival.[6,9,10] In several IEM, longer survival rates have unmasked a susceptibility to cancer, as seen in glycogen storage disease IV[11,12] (GSD IV [MIM 232500]). Hence, with accumulating experience, improved detection of IEM will allow surveillance and early intervention that could result in preferred outcomes of the IEM but could also lead to earlier detection of complications such as hepatocellular carcinoma (HCC) in tyrosinemia type 1 (caused by mutations in FAH [MIM 276700]) and citrin deficiency (MIM 603859).

Most metabolic disorders are inherited as autosomal-recessive conditions, and clinical problems are evident when the child inherits mutations in both alleles of the implicated gene. Typically, the heterozygous individual is healthy without evidence of disease because one normal allele is usually sufficient to maintain the rate of a reaction catalyzed by the encoded enzyme. However, having one mutant germline allele in every cell in the body as well as additional somatic events or second hits results in biallelic inactivation and somatic deficiency that can lead to carcinogenesis. Hence, individuals without a clinically evident metabolic disorder might demonstrate susceptibility to cancer as a result of inheriting single inactivating mutations in metabolic genes, for example, those encoding fumarate hydratase (*FH* [MIM 136850]) or subunits of

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DOI 10.1016/j.ajhg.2011.03.005.

succinate dehydrogenase[13] (*SDHA* [MIM 600857]). On the other hand, patients with IEM generally have near complete loss of enzyme function in every cell in the body because of two germline genomic changes, thus recurring patterns of cancers associated with specific IEM suggest a common genetic etiology and underscore the importance of specific metabolic dysregulation in the pathogenesis of cancer.

There are multiple ways by which IEM can predispose to cancers: accumulation of toxic metabolites, as seen with hereditary hemochromatosis (*HFE*-related HHC [MIM 235200] causing liver cirrhosis) and HCC; increased rate of mutations as seen in mitochondrial disorders; or by channeling metabolites to pathways preferentially used by cancer cells as aerobic glycolysis.[3] Hence, the dissection of a unique genetic and biochemical profile in a given IEM with a predisposition to a specific cancer offers an opportunity to understand the mechanisms of tumorigenesis of common cancers (Table 1).

Here, we review the prototypical biochemical features of IEM and their specific associated cancer predispositions. We discuss possible mechanisms leading to the specific cancer with an emphasis on the description of the dysregulated pathway. In addition, we give examples as to how the knowledge gained from specific metabolic changes led to the development of new therapeutic approaches to cancer.

IEM Pathogenesis and Cancer Mechanism

This section describes potential mechanisms involved in cancer development as related to specific IEM, which result in three different types of insults: (1) toxic accumulation of metabolites, (2) metabolite channeling, and (3) mitochondrial dysfunction. We hypothesize that the underlying metabolic disorder impacts the early stages of tumorigenesis/carcinogenesis as opposed to cancer progression. Hence we focus on the primary metabolic event that might lead to cancer initiation.

Accumulation of Toxic Metabolites

Enzyme deficiencies resulting in IEM generally cause accumulation of the enzyme's substrate and a deficiency of its downstream product (Figure 1).[14] In some cases, the accumulation of substrate is toxic, as seen in GSD, whereas in others the accumulation of toxic metabolite leads to an increase in oxidative stress and reactive oxygen species (ROS), as seen with iron accumulation in hemochromatosis.[15] In addition, accumulation of toxic metabolite could affect gene expression or cause a shift to alternative metabolic pathways, which could lead to tumorigenesis.[16] Because the liver is the organ in the body where most metabolic pathways are active, the toxic accumulation often results in cirrhosis-promoting HCC. Hence, although malignant liver tumors are an unusual cancer in children, accounting for approximately 1% of childhood malignancies,[17] HCC is one of the most common cancers observed with IEM.

HCC is seen in citrin deficiency;[18] tyrosinemia type I;[19] hemochromatosis;[20] porphyrias[21] (AIP [MIM 176000]); Wilson disease[22] (MIM 277900); Gaucher disease[23] (GD [MIM 230800]); GSD I[24] (MIM 232200), GSD III[25] (GSD III [MIM 232400]), and IV;[11] alpha 1-antitrypsin deficiency[26] (AATD [MIM 613490]); and MDDS.[27] The variety of different IEM predisposing to HCC suggests that it might not be directly linked to the genetic disorder per se but rather to the chronic and cumulative toxic damage causing liver fibrosis and cirrhosis eventually leading to HCC. Hence, cirrhosis could be one of the common mechanisms by which multiple IEM result in HCC.[28] In spite of this potential common pathway, it should be noted that each of the IEM described here has its own specific toxin accumulation that, in addition to cirrhosis and HCC, could promote other malignancies through different mechanisms. Of note, in a few IEM, for example in GSD I[29] and hereditary hemochromatosis, HCC has been described in the absence of cirrhosis, supporting the involvement of other mechanisms.[20]

Tyrosinemia type 1 results from the deficiency of fumarylacetoacetate hydrolase, which catalyzes the last step in the tyrosine degradation pathway.[30,31] Biochemically, FAH deficiency is characterized by accumulation of fumarylacetoacetate, maleylacetoacetate, succinylacetoacetate, and succinylacetone. The last compound is excreted in large quantities in the urine and is widely used as a screening and confirmatory test for FAH deficiency.[31] The disease is seen in high frequency in Saguenay-Lac St. Jean region of the province of Quebec, Canada and in Northern Europe. Clinically, tyrosinemia type 1 is characterized by acute and chronic liver failure with varying age of onset.[32] The risk of HCC is high, and about 40% of patients who survive beyond 2 years of age develop HCC.[28] It has been suggested that tumorigenic effects are linked to the ability of accumulated fumarylacetoacetate, maleylacetoacetate, and succinylacetone to disrupt thiol groups of proteins.[33] In addition, fumarylacetoacetate was reported to be a direct mutagen,[34] probably because of its alkylating properties. It has been demonstrated that nuclear factor erythroid-2-related factor 2 (Nrf2), a transcription factor participating in protection against oxidative stress, plays a central role in fumarylacetoacetate-induced liver damage.[19] A combination of ROS and the mutagenic properties of accumulated compounds in FAH deficiency results in primary liver cancer manifesting as HCC in 17%–37% of untreated patients.[35,36] Treatment with 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) prevents the accumulation of toxic metabolites and significantly decreases the incidence of HCC to $<2\%$ in these patients if administered in their first year of life.[37]

HFE-related hereditary hemochromatosis is an inborn error of iron metabolism caused by mutations in *HFE*-encoding (MIM 613609) hereditary hemochromatosis protein.[38,39] The gene product binds to the transferrin receptor and reduces its affinity for iron-loaded transferrin.

Table 1. IEM and Associated Tumors

IEM	Gene	Associated Tumors, Cancers and Syndromes	Proposed Mechanisms of Tumorigenesis	References
Tyrosinemia type 1	*FAH*	HCC	Fumarylacetoacetate, maleylacetoacetate, and succinylacetone can disrupt SH-groups of proteins and increase the damaging effects of ROS; fumarylacetoacetate can also be a direct mutagen	18, 26, 28-34
HFE-related hereditary hemochromatosis	*HFE*	HCC	Iron itself is carcinogenic and its accumulation increases oxidative tissue damage leading to carcinogenesis.	19, 35–42
Acute intermittent porphyria	*HMBS*	HCC	The final oxidation product of 5-aminolevulinate, 4,5-dioxovalerate might result in DNA alkylation and mutagenesis	20, 44–46, 49–50
PCT	*UROD*	HCC	Associated iron overload might result in carcinogenesis.	47–48
Wilson disease	*ATP7B*	HCC, cholangiocarcinoma, and abdominal adenocarcinoma	Dysregulation of the copper metabolism might result in the abnormal cytochrome c oxidase and mitochondria superoxide dismutase, thus altering ROS	51–56
GD	*GBA*	Hematologic malignancies, bone cancer	Abnormal stimulation of cells responsible for adaptive immunity by glucocerebroside-laden macrophages; increased stress of endoplasmic reticulum	57–61
AAT	*SERPINA1*	HCC	Accumulation of aggregated AAT with additional mitochondrial damage	64–71
Fabry disease	*GLA*	RCC	Chronic accumulative renal injury	72–76
GSD I, III, and IV	*G6PC* *SLC37A4* *AGL* *GBE1*	*HCC*	*Increased flux through the P5P pathway with altered DNA and RNA synthesis; diversion of fatty acid flux from beta-oxidation in the mitochondria into peroxisomes favoring production of H_2O_2; activation of proto-oncogenes through dysregulation of the insulin-glucagon-growth hormone trio*	*23–24, 26–27, 79–88*
SDH deficiency	*SDHA* *SDHB* *SDHC* *SHDD*	*Carney triad (paraganglioma, gastric stromal tumors and pulmonary chondromas), Carney-Stratakis syndrome; renal tumors and pheochromocytomas*	*Accumulation of succinate and fumarate cause a pseudohypoxia state resulting in an inhibition of HIF hydroxylases, leading to stabilization of activated HIF-1α and expression of HIF target proteins such as VEGF. Severe disturbance in the electron transport chain can lead to dysregulated ROS production*	*12, 15, 89–92, 96–106*
FH deficiency	*FH*	*Cutaneous leiomyomas, uterine leiomyomas, adrenocortical tumors, early-onset renal cell cancer, ovarian mucinous cystadenomas, Leydig cell tumors, leiomyosarcomas, and cerebral cavernomas*		*93–95*
L-2-hydroxyglutaric aciduria	*L2HGDH*	*Brain gliomas and glioblastomas, Wilms tumor, acute myeloblastic leukemia*	*L-2-hydroxyglutarate can promote Warburg effect and inhibition of HIF prolyl hydroxylase*	*107–110*
Isocitrate dehydrogenase deficiency	*IDH1* *IDH2*	*Brain gliomas and glioblastomas*	*Specific mutations in IDH1 and IDH2 might deplete α-KG by converting it into D-2-hydroxyglutarate, thus interfering with prolyl hydroxylases and inhibiting oxidative phosphorylation.*	*111–120*
Citrin deficiency (AGC2)	*SLC25A13*	*HCC*	*Increased uptake of thymidine promoting growth and inhibiting apoptosis secondary to unbalanced dNTP pools. Increased NADH/NAD^+ ratio stimulating fatty acid synthesis, steatosis and liver damage. Dysregulation of ROS.*	*121–126*

(*Continued on next page*)

Table 1. ***Continued***

IEM	Gene	Associated Tumors, Cancers and Syndromes	Proposed Mechanisms of Tumorigenesis	References
Deoxyguanosine kinase deficiency	***DGUOK***	**HCC**	**Unbalanced dNTP pools resulting in DNA mutations; increased generation of ROS;**	**131–140**
MPV17 deficiency	***MPV17***	**HCC**	**Mitochondrial DNA depletion resulting in the reduction of oxidative phosphorylation and dysregulation of the ROS biology**	**141**
MERRF	**tRNA-Lys (m.8344A > G)**	**Lipomas**	**Decrease in oxidative phosphorylation**	**142–145**

The table summarizes the IEM described in this review with the unique tumors that were found to be associated with each specific IEM. In addition, a proposed mechanism of tumorgenesis is suggested. Roman typeface indicates IEM related to accumulation of toxic metabolites; Italics indicate IEM related to metabolite channeling; Bold indicates IEM-related aberrant mitochondrial function.

Thus, loss of HFE function results in deposition of iron in most peripheral organs, including the liver. Hereditary hemochromatosis is the most common autosomal-recessive disorder in those of Northern European descent, affecting 1 in every 200–400 individuals.[39]Many individuals who carry two *HFE* mutations are healthy and show no iron overload. Affected individuals typically present between 40 and 60 years of age. In patients with clinically diagnosed *HFE*-related hemochromatosis, HCC is common and is responsible for 25%–45% of deaths.[20,40] Risk factors associated with HCC in *HFE*-related HHC include liver cirrhosis, infections with hepatitis B or C, alcoholism, and use of tobacco.[20] Consistent with the deposition of iron in all tissues, there might be increased risk of cancers in other tissues including melanoma, esophageal, ovarian, breast, hematologic, colorectal, and gastric malignancies.[41–43] In addition, HCC was described in HHC in the absence of cirrhosis and even after reversal of cirrhosis with therapy.[44] These reports suggest that iron accumulation could be involved in oxidative tissue damage directly leading to carcinogenesis.[45] Indeed, iron was shown to be carcinogenic in animal models, and moreover, the use of

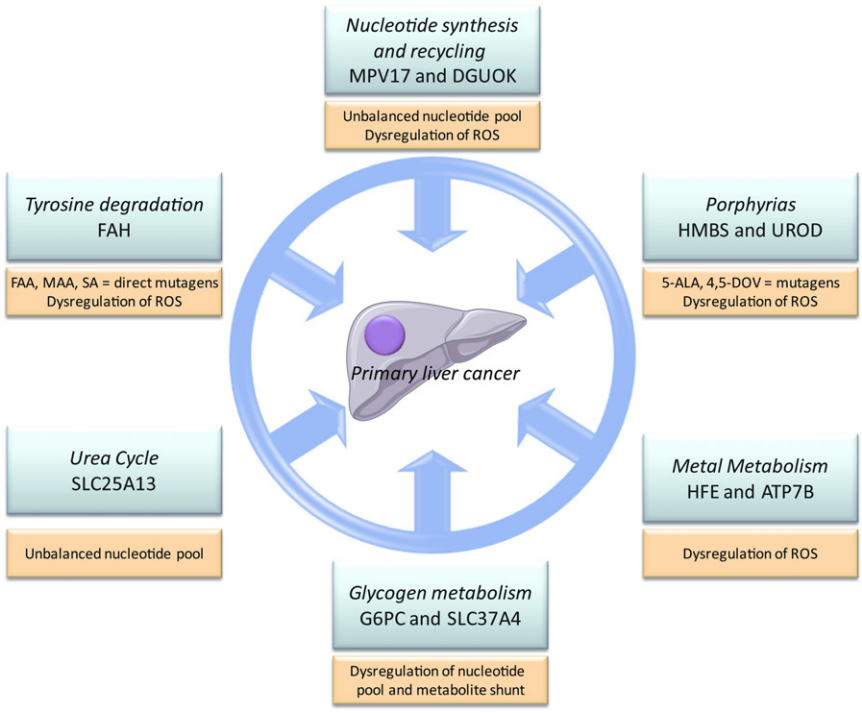

Figure 1. Diverse Mechanisms of Tumorigenesis in Primary Liver Cancer

Biochemically diverse metabolic disorders have primary liver cancer (HCC), which can occur even in the absence of discernible liver fibrosis or cirrhosis, as one of their later complications. Unique biochemical signatures in each metabolic disorder provide us with important clues regarding the dysregulation of major pathways instigating the transformation of healthy tissue into a tumor. Several common mechanisms emerge including (1) direct mutagenesis via interaction of intermediate metabolites with DNA, (2) imbalances in the nucleotide pool resulting in mitochondrial DNA damage, (3) dysregulation of ROS biology, and (4) diversion of the metabolic flux imitating Warburg effect. Although each mechanism alone is unlikely to be directly responsible for primary liver cancer, collectively they lower the threshold required for tissue transformation. The following abbreviations are used: 4,5-DOV, 4,5-dioxovalerate; 5-ALA, 5-aminolevulinate; ATP7B, ATPase Cu++ transporting beta polypeptide (Wilson disease); DGUOK, deoxyguanosine kinase; FAA, fumarylacetoacetate; FAH, fumarylacetoacetate hydrolase; G6PC, glucose-6-phosphatase, catalytic subunit (GSD Ia); HFE, hemochromatosis protein (*HFE*-related hereditary hemochromatosis); HMBS, hydroxymethylbilane synthase (dominant disease acute intermittent porphyria); MAA, maleylacetoacetate; MPV17, MPV17 mitochondrial inner membrane protein (hepatocerebral form of mitochondrial DNA depletion syndrome); ROS, reactive oxygen species; SA, succinylacetone; SLC25A13, solute carrier family 25, member 13 (mitochondrial aspartate-glutamate carrier protein, citrin deficiency); SLC37A4, solute carrier family 37, member 4 (G6PT, GSD Ib); UROD, uroporphyrinogen decarboxylase (familial PCT and hepatoerythropoetic porphyria).

phlebotomy in people without IEM reduced cancer risk.[45,46]

Disorders of heme metabolism encompass a large group of heritable conditions disrupting synthesis of heme from glycine and succinyl-CoA. Two hepatic forms of porphyria have been shown to be linked to increased risk of developing HCC, including acute intermittent porphyria and porphyria cutanea tarda (PCT).[47,48]

Acute intermittent porphyria is an autosomal-dominant hepatic porphyria due to deleterious mutations in *HMBS* (MIM 609806), encoding porphobilinogen deaminase.[49,50] There is incomplete penetrance, and the majority of *HMBS* mutation carriers are asymptomatic.[50] The deficiency of porphobilinogen deaminase in AIP is usually partial, resulting in clinically latent disease in the absence of aggravating factors. Clinical manifestations are seen when the heme-mediated repression of the upstream 5-aminolevulinic acid synthetase I (ALAS1) is relieved in the liver. This loss of feedback repression of ALAS1 usually occurs when there is an increased requirement for heme that is triggered by drugs inducing cytochrome P450 or ALAS1.[50] The restoration of this feedback loop by intravenous administration of heme results in the repression of ALAS1 and decrease in the synthesis of heme precursors thought to be responsible for clinical manifestations of this disease. Patients with AIP excrete significant amounts of 5-aminolevulinate and porphobilinogen in the urine, which can be used as a laboratory test for AIP diagnosis. HCC is the only cancer known to be associated with AIP.[21] A retrospective population-based study involving data on the population from northern Sweden determined that 27% of deceased AIP patients had evidence of HCC.[51]

PCT (MIM 176100) is the most common form of porphyria and is caused by a decrease in the activity of uroporphyrinogen decarboxylase, encoded by *UROD*[52,53] (MIM 613521). Affected individuals develop adult-onset light-sensitive dermatitis, often presenting with blisters in the sun-exposed areas of skin. Biochemically, PCT patients show increased uroporphyrin and heptacarboxyl porphyrin in liver tissues, plasma, and urine.[53] One sporadic (type I) and two familial (types II and III) forms have been described. Patients with PCT type I (sporadic) demonstrate profoundly decreased activity of uroporphyrinogen decarboxylase but show no deleterious mutations in *UROD*. Factors that can affect the enzyme activity include chronic infection with hepatitis C and HIV, alcohol and tobacco use, estrogens, toxins (such as hexachlorobenzene and halogenated polycyclic aromatic hydrocarbons), and the accumulation of iron. The relevance of this last factor can be observed in individuals with HHC, for whom the association between PCT, iron overload, and *HFE* mutations has been described.[54] PCT type II, an autosomal-dominant trait, is caused by deleterious mutations in *UROD*.

The initial insult leading to cirrhosis and HCC or to primary HCC in porphyria is thought to be alkylation of DNA by 4,5-dioxovalerate, which the final oxidation product of 5-aminolevulinate[55] and increases mutation rates. In addition to HCC, oral, stomach, lung, prostate, kidney, and bladder cancer have been shown to be significantly increased in porphyria patients,[48] as well as malignancy of the breast, pancreas, or colon and hence should be considered in patients presenting with an acute porphyric attack in their late 30s–40s.[56]

Wilson disease is an autosomal-recessive hereditary disorder of copper metabolism due to mutations in *ATP7B*[57,58] (MIM 606882). The gene encodes a copper-transporting ATPase involved in the outward cellular efflux of copper. It is most abundant in organs such as liver, kidney, and brain. In the liver, the protein is responsible for the efflux of copper into the bile; hence, the dysfunction of this transporter results in accumulation of copper in hepatocytes. Clinical manifestations of the disease include liver dysfunction and neurological and psychiatric symptoms.[58] HCC has been reported in approximately 6% of affected individuals.[59] Other malignancies include cholangiocarcinoma and abdominal adenocarcinoma without a known primary site. HCC in Wilson disease has been primarily found in patients treated with D-penicillamine with lower liver copper levels leading to the hypothesis that copper accumulation might be protective against hepatic carcinogenesis.[60] However, HCC has been described in an untreated Wilson patient as well as in rat models of Wilson disease (Long-Evans Cinnamon rats),[61] suggesting that copper's carcinogenic effect might be dosage dependent as well as influenced by other factors such as D-penicillamine dosage and age at diagnosis.[22,62] In addition, it has been suggested that copper's role in carcinogenesis could be related to its requirement for the proper functioning of cytochrome *c* oxidase and mitochondrial superoxide dismutase; thus, imbalance in copper pools could alter oxidative phosphorylation.[63]

GD is the most common lysosomal storage disease and results from the deficiency of β-glucocerebrosidase (GBA [MIM 606463]).[64] Patients with GD accumulate glucocerebroside in reticuloendothelial cells. Because of excessive accumulation of glucocerebroside in bone marrow, both cytopenia and bone lesions can occur. Hence, most common malignancies observed in GD are hematologic (lymphomas and multiple myeloma), together with bone-related cancers.[65,66] Chronic stimulation of the immune response by glucocerebroside-laden macrophages is one potential toxic mechanism.[67,68] Indeed, in GD there is an increased amount of circulating IgG and IgM antibodies, suggesting underlying lymphoproliferation. Others have proposed that leukocyte cellular function is directly impacted by the underlying enzyme deficiency rather than by chronic immune stimulation alone, which would also contribute to the increased risk of hematologic malignancy.[65] As for the bone-related tumors, it is thought that in GD, the occupancy of the bone marrow by abnormal cells disturbs the interaction between bone and bone marrow cells resulting in aberrant development and activity of the bone cells and causing anomalous bone remodeling predisposing to cancer.[69] Although rarely,

HCC has also been described in GD,[23,70] as well as breast cancer and melanoma, which were found to have a significant association with this lysosomal storage disease.[71]

AATD is an autosomal-recessive disorder caused by mutation in *SERPINA1*[72,73] (MIM 107400). The most common manifestation is emphysema, which becomes evident by the 30s to 40s, whereas a less common manifestation is liver disease, which occurs in children and adults and can result in cirrhosis, liver failure, and an increased risk of developing HCC.[26] Environmental factors, particularly cigarette smoking, greatly increase the risk of emphysema at an earlier age.[73] The diagnosis of AATD relies on the demonstration of a low plasma concentration of alpha 1-antitrypsin (AAT) and either observation of a deficient variant of the protein AAT by protease inhibitor (PI) typing or detection of mutations in both copies of *SERPINA1*. PI*Z, resulting from p.Glu342Lys; c.1024G>A [NM_000295.3 (we have numbered the mutation starting from the second ATG), is the most common deficiency allele and 95% of AATD are homozygous for it.[74] The AAT molecule is a serine PI that is predominantly produced in the liver. Its most important physiologic functions are the protection of pulmonary tissue from aggressive proteolytic enzymes and regulation of pulmonary immune processes. Hence, the basis for pulmonary disease in AATD is a reduced inhibition of leukocyte elastase in the lung, resulting in excessive destruction of the elastin in the alveolae.[75]

Homozygosity as well as heterozygosity for AATD has been shown to be related to the development of HCC.[26] In the heterozygous state, HCCs frequently develop in a noncirrhotic liver[76] and are often characterized by cholangiocellular differentiation. The chronic liver injury in AATD most probably results from accumulation of aggregated AAT in hepatocytes and bile ducts.[77] AAT aggregates induce proinflammatory pathways and can be a stimulus for hepatocarcinogenesis.[78] In addition, there is evidence for specific mitochondrial damage unique to AATD that causes mitochondria autophagy, increasing the liver damage, cirrhosis, and the predisposition to HCC.[79]

Similar to the finding that chronic toxic metabolite exposure in the liver leads to HCC, bilateral renal cell carcinoma (RCC) has been described in long-term Fabry disease (MIM 301500).[80,81] Fabry disease is an X-linked inborn error of glycosphingolipid catabolism and results from deficient or absent activity of the lysosomal enzyme alpha-galactosidase A[82] (GLA [MIM 300644]). This enzymatic defect leads to the systemic accumulation of globotriosylceramide (Gb3) and related glycosphingolipids in the plasma and cellular lysosomes of vessels, nerves, tissues, and organs throughout the body.[82] Manifestations of Fabry disease include serious and progressive impairment of renal and cardiac function. In addition, patients experience pain, gastrointestinal disturbance, transient ischemic attacks, and strokes. Additional effects on the skin, eyes, ears, lungs, and bones are often seen.[83] Despite the fact that the disease is X linked, heterozygous females can present with clinical complications requiring medical interventions and hence should not be considered carriers.[84]

Two independent groups describe RCC in Fabry patients.[80,81] Although no clear etiologic relationship between the metabolic and neoplastic phenotype has been found yet, the common observation of RCC in Fabry patients suggests this association to be more than coincidental.[80] Importantly, the likelihood of bilateral multifocal RCC being an isolated finding is low and is typically associated with known cancer susceptibility syndromes such as familial RCC, Von Hippel-Lindau disease, polycystic kidney disease, and tuberous sclerosis.[80,81]

Alteration of Metabolite Channeling

Cancer-associated metabolic programs include increased rates of oxygen independent glycolysis, glutaminolysis, and fatty acid synthesis (Figure 2A).[85,86] These functional changes in cell metabolism give transformed cells competitive advantages for rapid proliferation. Glycolysis and glutamine oxidation provide glucose and glutamine as carbon sources for energy production and anabolism, whereas the fatty acid synthesis provides lipids necessary for membrane production and post translational modifications of proteins. For support fatty acid biosynthesis to occur, there is an additional requirement for NADPH generated by the pentose phosphate (P5P) pathway. Hence, there is a shift in the glycolytic intermediate products intended to increase production of NADPH, lipid synthesis, and nucleic and amino acids, all of which would serve as building blocks to support rapid proliferation.[85,86] Some overlapping metabolic features can be seen in patients with GSDs and disorders of the tricarboxylic acid cycle:[87] increased anaerobic glycolysis, increased flux through the P5P pathway, and alteration in the fatty acid metabolism.

GSD I is an inborn error of metabolism characterized by abnormal glycogen metabolism due to deficiency of glucose-6-phosphatase complex[88] (G6PC [MIM 613742]). GSD I patients are unable to mobilize the glycogen stores to offset fasting. They present with hepatomegaly, fasting hypoglycemia, accumulation of glycogen, lactic acidosis, hyperuricemia, and disturbance of the lipid metabolism. Approximately 80% of cases (GSD Ia) result from mutations in *G6PC* encoding glucose-6-phosphatase-α catalytic unit. The remaining cases (GSD Ib) are due to mutations in *SLC37A4* encoding glucose-6-phosphate transporter (G6PT [MIM 602671]). Sixteen percent of GSD Ia or GSD Ib patients between ages 2 and 30 years develop hepatic adenomas.[89] These adenomas are frequently precancerous and can carry up to a 10% risk of malignant transformation.[90] The time between the diagnosis of liver adenomas and HCC ranges from 0 to 28 years, and the age of HCC diagnosis ranges between 19 and 49 years.[24]

GSD III is an autosomal-recessive disorder due to the deficiency of glycogen debranching enzyme encoded by *AGL*[91] (MIM 610860). The enzyme possesses two catalytic

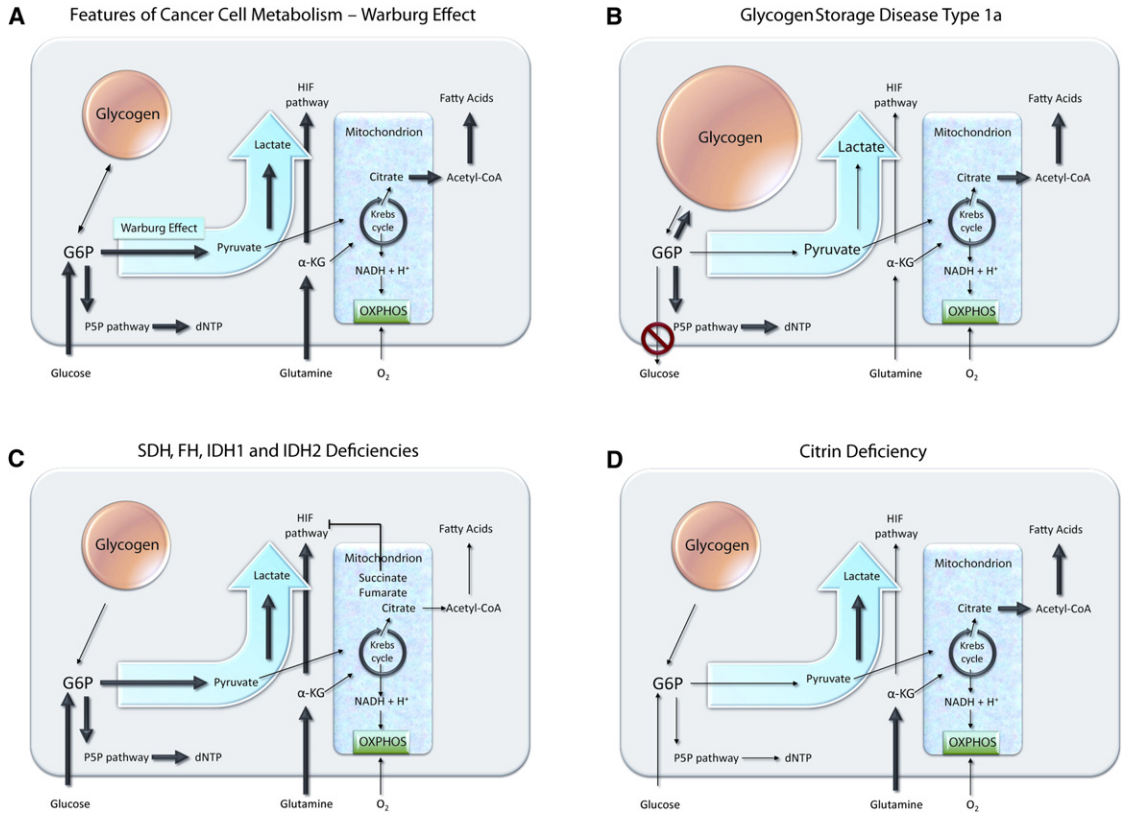

Figure 2. Warburg Effect and Metabolic Disorders with Features of Metabolite Shunting
(A) The observed Warburg effect in cancerous cells is a metabolic phenomenon of aerobic glycolysis, that is, diversion of glucose via pyruvate to lactate even in the presence of abundant oxygen. In addition to the Warburg effect, other important features of metabolism in cancerous cells include increased synthesis of nucleotides to synthesize DNA, diversion of citrate to cytosolic acetyl-CoA for fatty acid synthesis, and increased need for glutamine, a precursor for alpha-ketoglutarate (α-KG), intrinsically linked to the HIF-pathway.
(B) The sum of metabolic derangements observed in GSD Ia, where the inability to convert G6P to glucose results in increased shunting of G6P to P5P pathway. G6P is also used as a substrate for fatty acid and triglyceride synthesis. Oversupply of lactate and pyruvate through Cori cycle and hepatic gluconeogenesis could result in alteration of the ATP/ADP and NADH/NAD^+ ratios in favor of tumorigenesis.
(C) Metabolic abnormalities highlighting the role of Krebs cycle intermediates—succinate, fumarate, and α-KG—in the regulation of the HIF-dependent pathway. Impaired function of Krebs cycle might adversely affect oxidative phosphorylation and thus lead to cell-autonomous shunting of glucose to pyruvate and lactate.
(D) Citrin deficiency is characterized by increased metabolism of simple carbohydrates resulting in high NADH/NAD^+ and lactate/pyruvate ratios, which can also be observed under the Warburg effect. Fatty liver in citrin deficiency implies increased flux of citrin via acetyl-CoA for fatty acid synthesis.

properties: amylo-1,6-glucosidase (EC 3.2.1.33) and 4-alpha-glucanotransferase (EC 2.4.1.25). The glucosidase and transferase activities are mediated by two structurally separate catalytic centers, but both are required for normal functioning of the enzyme.[92] In contrast to GSD I, patients affected by GSD III can present with liver and muscle phenotype and appear to be at increased risk for liver fibrosis, often progressing to cirrhosis. Multiple reports demonstrate the association between GSD III and HCC is usually preceded by liver cirrhosis but not always by hepatic adenomas, suggesting that both adenomas and cirrhosis can contribute independently toward malignant transformation.[25,93] Although most GSD III patients have both liver and muscle involvement, for reasons yet to be elucidated, tumors do not appear to arise from the muscle tissue.

Yet another form of glycogen storage disorder that has been linked to primary liver cancer is GSD IV. The disease results from a deficiency of glycogen branching enzyme because of mutations in *GBE1*[94] (MIM 607839). GSD IV is a clinically heterogeneous condition and can affect liver, muscle, and the central nervous system. Branching enzyme deficiency leads to accumulation of abnormally structured glycogen with longer branches and fewer branching points, thus showing similarity to amylopectin, found in plant starch.

HCC has been reported in GSD I,[95] III,[25] and IV.[11,12,96] In GSDs, the mechanism related to the development of HCC has been frequently linked to liver cirrhosis. Cirrhosis from any cause appears to be the common pathway by which several risk factors exert their hepatocarcinogenic effect.[28] However, GSD I is not associated with cirrhosis. Approximately 75% of patients with GSD I develop liver adenomas despite apparent normoglycemia.[29] In fact, liver adenomas are the most common indication for liver transplantation in GSD I.[97] HCC is estimated to occur in 10% of patients

with GSD I,[98] most often as a result of malignant transformation of these adenomas. This clearly demonstrates HCC risk in the absence of cirrhosis in this IEM. However, adenomas are not a required precursor lesion because there have been cases of GSD I with primary HCC[24] and cases of HCC in children younger than 1 year of age.[95]

In addition to hypoglycemia, there are additional unique biochemical characteristics seen in GSD I patients: lactic acidosis, hyperlipidemia, and hyperuricemia.[95] The deficiency in G6Pase complex increases glycolysis and as result there is an increase in lipolysis leading to enhanced production of acetyl-CoA that leads to stimulation of lipid synthesis and inhibition of fatty acid oxidation. Liver steatosis occurs because of these imbalances in fatty acids metabolism.[99] The increase in the glycolytic rate also leads to increased flux through the P5P pathway causing altered DNA and RNA synthesis. The high glycolytic and fatty-acid synthesis rates are among the classical changes thought to predispose cells to malignant transformation.[85,86] In addition, several other mechanisms responsible for development of hepatic adenomas in GSD I and their potential transformation into HCC have been proposed including chromosomal alterations and activation of proto-oncogenes through dysregulation of the insulin-glucagon-growth hormone trio[29,100] (Figure 2B).

Homozygous mutations in the genes encoding the subunits of the succinate dehydrogenase enzyme (SDH), including *SDHA* and FH, result in an autosomal-recessive IEM.[101] Common features of these inherited enzyme deficiencies include early-onset encephalomyopathy, hypotonia, epilepsy, and failure to thrive.[101] These recessive conditions are rare and patients have a shortened life span, thus, it is not known at present whether affected individuals are at a higher risk for cancer. On the other hand, heterozygous mutations in *FH*, *SDHB* (MIM 185470), *SDHC* (MIM 602413), *SDHD* (MIM 602690), and the recently described *SDHA* result in the cancer phenotypes that are inherited in an autosomal-dominant manner.[13] The heterozygous nature of the mutations results in near normal metabolic function in somatic cells such that patients don't demonstrate IEM classic clinical features. Instead, somatic loss of the remaining wild-type allele—often associated with loss of heterozygosity of nearby polymorphic markers—will result in tissue-specific complete loss of function of the affected enzyme and loss of their tumor suppressor activity in somatic tissues.[102,103]

Inheritance of heterozygous *FH* mutations predisposes to multiple cutaneous leiomyomas, uterine leiomyomas, adrenocortical tumors, and renal cell cancer, collectively referred to as HLRCC.[104,105] Skin leiomyomas due to germline *FH* mutations typically present between ages 20–40 years, and there is a slight predilection in female patients. Female carriers of *FH* mutations can present with early-onset uterine leiomyomas, often requiring hysterectomy. Mutations in *FH* also predispose to aggressive early-onset RCC. Other malignancies that have been associated with mutations in *FH* include ovarian mucinous cystadenomas, Leydig cell tumors, leiomyosarcomas, and cerebral cavernomas.[106]

SDH is a heteroligomer containing subunits A, B, C, and D. Mutations in genes encoding all subunits have been linked to susceptibility to paragangliomas, which could be non-syndromic and syndromic forms. The syndromic forms include Carney triad (paraganglioma, gastric stromal tumors, and pulmonary chondromas) and Carney-Stratakis syndrome.[16,107] The difference between these two syndromes is the inheritance mode, which is autosomal dominant in Carney-Stratakis syndrome, whereas the Carney triad can result from de novo point mutations and genomic rearrangements, the most frequent being deletion of the long arm of chromosome 1 (1q), which includes *SDHC*.[107]

The spectrum of tumors in SDH deficiency includes hereditary paragangliomas, gastric stromal tumors, renal tumors, and pheochromocytomas.[108,109] Cancer-predisposing mutations in genes encoding the *SDHA*, *SDHB*, *SDHC*, and *SDHD* subunits of mitochondrial complex II have all been reported.[110] Mutations in *SDHB* are more likely to result in metastatic paragangliomas.[111] In the US, germline mutations in *SDHB* and *SDHD* account for approximately 70% of familial cases and approximately 8% of apparently sporadic cases of head and neck paragangliomas.[112]

Multiple studies show that severe accumulation of succinate and fumarate cause a pseudohypoxia state resulting in an inhibition of hypoxia-inducible factor (HIF) hydroxylases and leading to stabilization of activated HIF-1α and expression of HIF target proteins such as vascular endothelial growth factor (VEGF).[113] HIF activation enhances glycolysis, decreases the flux through the Krebs cycle, and promotes transformation of certain cells as clear cell renal cells.[16] The association of mutations in *VHL* (associated with HIF1-alpha dysregulation), *FH, SDHB* and *SDHD* with renal cancer[109] is consistent with the notion of renal epithelial cells are particularly susceptible to HIF-induced transformation,[16] supporting the model that renal cancer is a disease of cell metabolism.[114] Another possible mechanism by which HIF causes renal cancer is that both FH and SDH deficiencies result in severe disturbance in the electron transport chain leading to higher ROS production.[112,115,116] The reason why *SDH* mutations are linked especially to paragangliomas is not clear but could be related to the embryonic origin of paragangliomas when an excess number of neuroblasts is generated, and SDH deficiency could prevent necessary apoptosis[117] (Figures 2C and 3).

L-2-hydroxyglutaric aciduria (MIM 236792) is caused by mutations in the gene encoding L-2-hydroxyglutarate dehydrogenase (MIM 609584), which localizes to mitochondria.[118] Patients with L-2-hydroxyglutaric aciduria are characterized by progressive ataxia, mental deficiency, encephalopathy, subcortical leukoencephalopathy, and cerebral atrophy. The disease is inherited in an autosomal-recessive manner and can be diagnosed by the presence of elevated concentrations of L-2-hydroxyglutaric

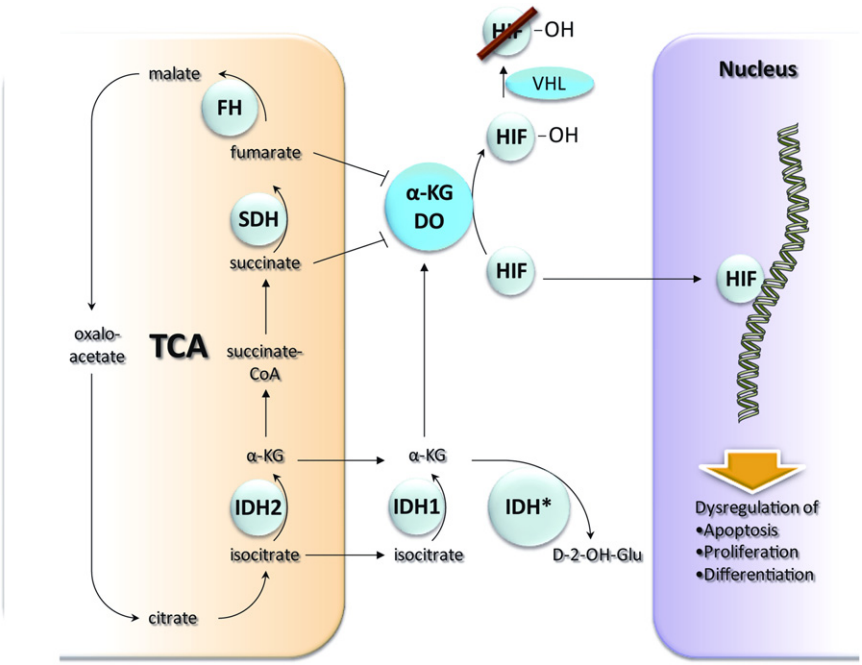

Figure 3. The Role of Tricarboxylic Acid Cycle Intermediates in the Regulation of HIF Function

Deficiencies of FH and SDH in the tricarboxylic acid[87] cycle results in the accumulation of fumarate and succinate, respectively. Accumulation of fumarate and succinate results in inhibition of the α-KG-dependent dioxygenases (α-KG DO) responsible for hydroxylation of the HIF, which further impairs the recruitment of VHL protein necessary in the HIF degradation. Isocitrate dehydrogenase deficiency results in the depletion of α-KG, thus decreasing the activity of some α-KG DO. Some unique mutant alleles, IDH2* and IDH1*, which are the result of modification of the arginine at position 140 or position 172 in IDH2 (NP_002159.2) or the arginine at position 132 of IDH1 (NP_005887.2), confer the enzymes a novel function of converting α-KG into D-2-hydroxyglutarate (D-2-HGA). This conversion results in depletion of α-KG and an increase in the concentration of D-2-HGA, a unique oncometabolite and a possible competitive inhibitor of α-KG-dependent enzymes. Collectively, these changes result in dysregulation of the HIF-induced apoptosis, proliferation, and differentiation underlying cancer mechanisms.

acid in plasma, urine, or cerebrospinal fluid[119] or by brain MRI findings and molecular diagnosis. The association between L-2-hydroxyglutaric aciduria and the development of CNS tumors has been reported by several groups.[120,121] Moreover, a child with L-2-hydroxyglutaric aciduria and Wilms tumor has been recently reported, potentially expanding the tumor spectrum in this metabolic disorder.[122] L-2-hydroxyglutaric aciduria patients who develop brain tumors can present with sudden and unexpected worsening of symptoms; however, close clinical follow up and MRI evaluation are recommended because it is difficult to identify a brain tumor in the presence of severe underlying white matter abnormalities.[121]

This association is an example of how difficult it can be to characterize the underlying molecular mechanism leading to tumorigenesis. On the one hand, as described below, L-2- hydroxyglutaric acid by itself was determined to be as an oncometabolite when it accumulates in high doses. On the other hand, L-2- hydroxyglutaric acid can reduce the availability of a Krebs cycle intermediate, alpha-ketoglutarate (α-KG), altering the mitochondrial function and shifting the cells to glycolysis as a preferential pathway for energy.

Until recently, no data were available to explain why L-2-hydroxyglutaric aciduria predisposes the brain to oncogenesis. In 2008, it was found that 70% of subjects with grade II and grade III gliomas, 12% of subjects with glioblastomas, and 15%–20% of adults with acute myeloid leukemia harbor somatic mutations in the isocitrate dehydrogenase 1 (*IDH1* [MIM 147700]) and 2 (*IDH2* [MIM 147650]).[123] Both IDH1 and IDH2 are NADP+-dependent enzymes and catalyze the oxidative decarboxylation of isocitrate into α-KG, a oxalosuccinate is formed as an intermediate product.[124] IDH1 localizes to the cytoplasm and peroxisomes, whereas IDH2 is found in the mitochondria.[125] *IDH1* and *IDH2* were first thought to be tumor suppressor genes for which loss of function result in an increased level of HIF (Figure 2C). However, unlike most tumor suppressor genes, which are characterized by dispersed mutations affecting both alleles, mutations found in *IDH1* and *IDH2* were monoallelic and confined to a single residue in the enzyme's active site.[126,127] This led to the discovery that the mutations in *IDH1* and *IDH2* are specific and cause these enzymes to acquire the ability to convert α-KG to 2-hydroxyglutarate.[128] Thus, IDH, instead of generating α-KG, acts on the available α-KG to generate 2-hydroxyglutarate.[129,130] As of yet, it is unclear whether there is also a decrease in α-KG contributing to pathogenesis.[130,131] The debate is ongoing whether *IDH1* and *IDH2* possess both oncogene and tumor suppressor activities. In the interim, 2-hydroxyglutarate has been designated as an oncometabolite.[126] It is thought that 2-hydroxyglutarate reduces HIF levels and in addition potentially affects mitochondrial function and gene expression, promoting the Warburg effect and directing cells toward aerobic glycolysis.[124]

These findings have important implications for management of patients. Mutated *IDH1* and *IDH2* are an important prognostic factor. The mutant proteins result in consumption of NADPH with sensitization of tumor cells

to irradiation and chemotherapy and, hence, increases patients' survival.[132] In addition, future cancer drug development will focus on developing antibodies specific for the IDH mutation, which might be given in conjunction with supplementation of α-KG[124,132] (Figure 2C).

Citrin deficiency is an autosomal-recessive condition caused by mutations in *SLC25A1*.[133] The protein named Citrin is a mitochondrial aspartate glutamate transporter (AGC2 [MIM 600637]). Depletion of cytosolic aspartate impairs handling of citrulline by argininosuccinate synthase (ASS), leading to accumulation of citrulline in plasma as well as in tissues and to impaired ureagenesis.[134] Clinically, citrin deficiency can manifest itself in two forms: citrullinemia type 2 (CTLN2 [MIM 603471]) and neonatal intrahepatic cholestasis (NICCD [MIM 605814]). CTLN2 is distinct from citrullinemia type 1 caused by ASS deficiency (IM 215700), although CTLN2 usually causes a secondary decrease in ASS activity. Although CTLN2 was initially observed in patients whose ancestry was East Asian (mainly Japanese), more recent studies suggest that citrin deficiency is a panethnic condition.[135] The adult-onset disease, CTLN2, is characterized by hyperammonemia, encephalopathy, liver fibrosis, and steatosis usually occurring between ages 20 and 50 years. Biochemically, CTLN2 patients show moderately elevated plasma citrulline and in some cases markedly increase alpha-fetoprotein levels. Multiple case reports established an epidemiological link between citrin deficiency and HCC.[18] HCC is typically diagnosed in CTLN2 patients between 21 to 66 years of age. Typical liver findings at the time of HCC diagnosis include fibrosis, steatosis, and cirrhosis, which could all contribute to HCC development. However, there have been several cases of CTLN2 and HCC without liver cirrhosis[136] in which the etiology of the HCC was attributed to nonalcoholic fatty changes in the liver (Figure 2D). It is thought that citrin deficiency leads to blocking of the malate aspartate shuttle, increasing the ratio of cytosolic nicotinamide adenine dinucleotide to oxidized nicotinamide adenine dinucleotide (NADH/NAD^+), which causes an increased lipid synthesis. The high level of fatty acids leads to steatohepatitis that could in turn cause fibrosis and liver cirrhosis-promoting HCC.[137] Another hypothesis is that citrin deficiency behaves like a mitochondrial disorder, and damage caused by increased oxidative stress in the liver might lead to HCC. As during the precancerous period, the levels of 8-hydroxy-2-deoxyguanosine, a biomarker of oxidative stress, increase in the urine.[138]

In addition, because one of the unique biochemical findings in citrin deficiency is hypercitrullinemia; it was suggested that excess amounts of citrulline are tumorigenic because its accumulation leads to an increased uptake of thymidine by the hepatocytes enhancing DNA synthesis and promoting proliferation.[136–139] However, the lack of reports of HCC in patients with classic citrullinemia (ASS deficiency) would argue against citrulline being the primary carcinogenic agent.

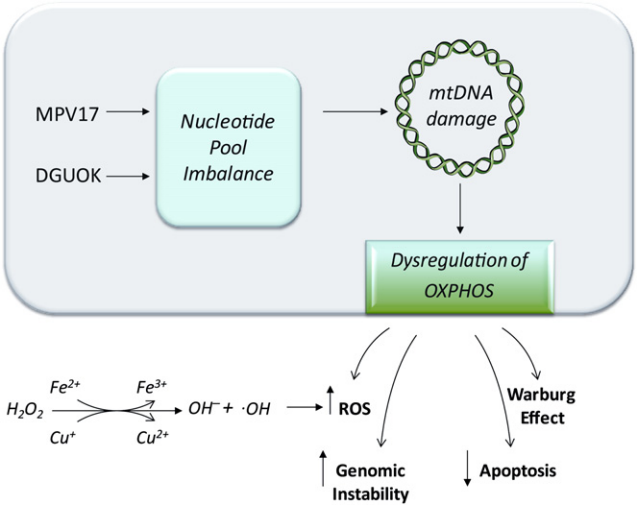

Figure 4. Mitochondria as the Target and Amplifier of the Tumorigenic Biochemical Signals
Mutations in MPV17 and DGUOK result in imbalance of the nucleotide pool thus increasing mtDNA instability. Accumulating mtDNA damage eventually culminates in the impairment of oxidative phosphorylation (OXPHOS). Impaired OXPHOS causes dysregulation of the ROS biology, propagates further genomic and mtDNA instability, decreases apoptosis, and creates the biochemical environment reminiscent of Warburg effect. Metabolic disorders resulting in accumulation of copper (Wilson disease) and iron (hemochromatosis) increase ROS presumably through Fenton reaction.

Aberrant Mitochondrial Function

Decreased mitochondrial oxidative phosphorylation is one of the hallmarks of cancer (Figure 4).[140] Here, we will focus on IEM generating increased ROS and imbalances in the mitochondrial deoxynucleotide (dNTP) pool as a primary insult predisposing to cancer formation. Imbalance of the dNTP pools has been shown to induce genomic instability in multiple ways, including base substitutions and enhanced susceptibility to breakage as well as promotion of chromosomere re-arrangement, breakage, and loss,[141,142] all of which could predispose to cancer. Increased ROS has also been linked with DNA damage through oxidation of nucleotide bases[143,144] promoting cell growth and hence contributing to carcinogenesis.[143]

mtDNA depletion syndromes (MDDSs) represent a group of autosomal-recessive disorders resulting from mutations in the nuclear-encoding genes participating in biogenesis of mtDNA and homeostasis of the mitochondrial nucleotide pool[140]. Although data are limited, a review of 76 patients with deoxyguanosine kinase (dGK) deficiency caused by mutations in *DGUOK* (MIM 601465) demonstrated that two subjects developed HCC.[145] In addition, mutations in *MPV17* (MIM 137960), which encodes the mitochondrial inner membrane protein MPV17 and has been found to cause hepatocerebellar mtDNA depletion, were also found to be associated with HCC.[146,147] Unlike dGK, the exact function of MPV17 is unknown.[148]

The mechanism of HCC tumor development in dGK deficiency could be related to neonatal hepatitis or

cirrhosis. [149] In addition, it is interesting to note that analysis of sporadic HCC samples demonstrates somatic mtDNA depletion.[25, 27,150] Depletion of mtDNA from cancerous cells enhances the tumorigenic phenotype in vitro and in xenograft models of human breast cancer.[151] Both human breast cancer and prostate cancer cells depleted of mtDNA were found to exhibit a more aggressive cancer phenotype.[152] In addition, it has been demonstrated in animal models that unbalanced mitochondrial dNTP pools are mutagenic,[153,154] and it can be hypothesized that this could be the mechanism responsible for the development of HCC in dGK disease. It has been suggested that MPV17 plays a role in controlling mtDNA maintenance and oxidative phosphorylation activity in mammals and yeast. The *SYM1*, the ortholog of *MPV17* in *Saccharomyces cerevisiae*, functions in the cellular response to metabolic stress and in maintaining mtDNA integrity and stability.[155] Thus, in the absence of functional MPV17, there is a risk for increased generation of ROS that could stimulate the development of HCC by itself or lead to increased mutation rate.

Another example of mitochondrial disorder associated with tumor growth is myoclonus epilepsy and ragged-red fibers (MERRF [MIM 545000]) syndrome, a progressive mitochondrial encephalopathy characterized by myoclonic seizures, ataxia, dementia, and hypotonia.[156] It results from the mtDNA point mutation that changes a highly conserved adenine to guanine at nucleotide 8344 in the mitochondrial tRNA for lysine, (*tRNA-Lys* [MIM 590060], m.8344A>G).[157,158] Familial multiple lipomas symmetrically located around the neck have been described in several patients with MERRF[159,160] and in carriers of the m.8344A>G mutation. The finding of a high proportion of mutated mtDNA, together with abnormal mitochondria in lipomas from carriers, suggest that the appearance of the lipoma is a primary manifestation in adipose tissue.[157] It could be that the defect in oxidative phosphorylation might interfere with normal adipocyte maturation.[157]

Therapy Targeting Metabolic Pathways Dysregulated in Cancer Cells

In this section, we describe how the recognition of metabolic abnormalities in cancer cells deepened our understanding of tumorigenesis, allowing the development of new cancer treatments. We will discuss two examples: (1) ASS deficiency in tumors as a concept of nutrient-metabolite deprivation and (2) targeting drugs for mitochondrial dysfunction in tumor cells putting them at survival disadvantage through mechanisms mediated by ROS.

A noncancerous eukaryotic cell is able to adapt to nutritional deprivation by breaking down its reserves and synthesizing essential components to survive until nutritional status improves. In contrast, cancer cells are unable to synthesize certain amino acids[161] and are thus dependent on the supply of these amino acids from external sources. One can exploit this nutritional difference to explore new therapeutic approaches. This strategy was applied to slow the growth of tumor cells through depletion of arginine. In humans, arginine is a nonessential amino acid because it can be synthesized from citrulline in two steps by using ASS and argininosuccinate lyase (MIM 608310) enzymes. However, in vitro experiments demonstrated that of 80% of primary and established cancer cell lines tested, the cells were unable to grow in media deprived of arginine because of their inability to convert citrulline to arginine, making arginine an essential amino acid.[162] In parallel, ASS deficiency was demonstrated in several human cancers including melanoma, HCC and prostate carcinoma.[163] In addition it was found that ASS deficiency is significantly associated with increased lymphatic dissemination of esophageal carcinoma[164]and with osteosarcoma lung metastasis.[165] Hence, arginine depletion could have beneficial effect specifically in those tumors with low ASS expression and poor prognosis. Much older studies demonstrated that *Mycoplasma hominis* can deplete arginine from tumor cells by using an enzymatic pathway not present in the mammalian cell host, i.e., arginine deiminase (ADI), which converts arginine to citrulline allowing *M. hominis* to target and kill specifically human tumor cell lines.[166–168] Of interest, this pathway is also involved in the generation of ATP when two other enzymes, ornithine transcarbamoylase and carbamate kinase, act in concert with ADI in *M. hominis*.[169] Recently, these observations made half a century ago, led to the development of a new treatment approach.[170] Pegylated ADI depletes intracellular arginine in cancer cells, which have an acquired ASS deficiency and thus are unable to resynthesize arginine from citrulline.[171] Phase I and II clinical trials with pegylated ADI led to a 47% response rate in patients with unresectable HCC, and a 25% response rate in patients with metastatic melanoma.[172,173] Additional cancer types are in the pipeline for clinical treatment trials. Hence, understanding of metabolic pathways differentiating the survival mechanism of normal cells from cancer cells has enabled the development of a specific therapy with beneficial clinical outcomes.

The mitochondria are key participants in ATP production, cell death, and ROS homeostasis. Multiple studies suggest that cancer cells' ability to grow and proliferate under increased ROS stress might be because of mitochondrial dysfunction leading to decreased apoptosis[143,174] Hence, therapeutic strategies could rely on three main differences between the mitochondria function in cancer versus normal cells: the higher glycolytic activity, the increase in ROS, and the differential regulation of apoptosis observed in cancer cells.[175,176] Indeed, defects in mitochondrial function have long been suspected to contribute to the development and possibly progression of cancer. As described in the introduction, Warburg described in 1956 the unique tumor phenotype where even in the presence of oxygen, tumor cells preferentially metabolize glucose to lactic acid and not to ATP as occurs in normal cells.[2] This phenomenon commonly referred to as the Warburg effect suggests that mitochondrial

alterations could provide unique therapeutic targets in various cancer types. In the late 1970s, the role of mitochondria in cancer was bolstered by the discovery that hepatic tumors (hepatomas) express hexokinase (EC 2.7.1.1) type II, (HK-2 [MIM 601125]), whereas normal hepatic cells express hexokinase IV, HK-4, also known as glucokinase (GCK [MIM 138079]). These two hexokinases differ in their cellular localization and in their enzyme kinetics, i.e., HK-2 interacts with the outer mitochondrial membrane protein voltage-dependent anion channel (VDAC) also known as mitochondrial porin,[177] and has a 250-fold lower K_M for glucose meaning, much higher glucose affinity relative to the cytosolic HK-4. HK-2 localization gives it preferential access to mitochondrial generated ATP enhancing the rate of glycolysis as well as protection from feedback inhibition by its product glucose-6-phosphate (G6P). Hence, tumors overproducing HK-2 can produce G6P at high rate providing support for uncontrolled tumor proliferation.[178–180] This scientific discovery led quickly to clinical applications. By using a deoxy analog of glucose (2-deoxy-D-glucose)that can be phosphorylated by HK-2 but not metabolized further, and that had been labeled with the positron emitter 18F, it became possible to image cancers for the first time in human patients.[181] This imaging technology now widely known as *p*ositron *e*mission *t*omography (PET scan) is utilized worldwide in humans for detecting all types of malignant tumors and monitoring their treatment.[181] When it was later shown that the complex HK-2-VDAC also has anti-apoptotic effects,[182] it was logically hypothesized that targeting tumor glycolysis by silencing HK-2 or disruption of its interaction with VDAC, would halt tumor proliferation and induce apoptosis.[183] Indeed, small molecules analogs of pyruvate targeting HK-2 have been shown to be highly effective in animal cancer models and are now in preclinical trials.[184]

Changes in mtDNA content and mtDNA mutations leading to altered expression and activity of respiratory chain subunits, have also been shown to increase tumorigenesis in part because of an increased ROS production.[116] A clear demonstration that a mtDNA mutation in cancer cells could be functionally significant came from a study that introduced a known pathogenic mtDNA mutation into a prostate cancer cell line, resulting in increased tumorigenicity in nude mouse transplantation studies.[185,186] There are several differences between the nuclear and mtDNA that might be responsible for increased instability in tumors including: the close proximity between the mtDNA and the site of ROS production, mtDNA doesn't have introns or histones, and mtDNA has limited DNA repair machinery. Undoubtedly, increased ROS production that occurs with mitochondrial dysfunction is an important contributor to tumorigenesis,[187] resulting in a vicious cycle in which mtDNA mutations lead to more ROS production, which in turn generates more mtDNA alterations that lead to increased genomic instability and often to cancer.[143]There are multiple other ways by which increased ROS contributes to tumorigenesis including: causing changes in nuclear gene expression,[188] acting as a stimulant in the proliferative response[189,190] by affecting the conformation and phosphorylation status of key proteins in signaling pathways as MAPK[191] Akt[192,187] and by influencing DNA- binding ability of various transcription factors as Ets-1[193]and p53.[194] With the increased ROS, cancer cells are more dependent on antioxidant defense and it was indeed demonstrated that human leukemia and ovarian cancer cells are more sensitive to superoxide dismutase inhibition than normal cells.[175] Finally, the apoptotic regulator Bcl-2 localizes to the mitochondrial outer membrane where it prevents the release of cytochrome *c* and other proapoptotic proteins.[176] Bcl-2 proteins are overexpressed in many cancers as the result of either translocations or dysregulation contributing to tumorigenesis. Indeed, Bcl-2 inhibitors have shown to induce cancer cell apoptosis by promoting cytochrome *c* release and initiating tumor regression in mice.[195,196]

In summary, somatic mutations of either nuclear encoded mitochondrial genes or mtDNA mutations and mtDNA depletion are frequent in solid and hematological malignancies.[197] Thus, as suggested by Warburg, mitochondrial alterations provide unique therapeutic targets in various cancers.

Interaction of IEM and Cancer Therapy

IEM complicated by cancer present a unique clinical challenge because there is an increased risk for toxicity resulting from the additional interaction between chemotherapy and the IEM. This gene X environment interaction is characterized at the simplest level by the catabolic state associated with chemotherapy that would worsen or potentially destabilize any patient with an IEM. Alternatively, it might be specific for the underlying enzyme derangement and the therapy employed. Few cases have been described to date but undoubtedly more will emerge with the increased survival of patients with IEM. Understanding the metabolic abnormality could prevent drug toxicity and increase treatment benefits.

As most chemotherapy drugs are metabolized in the liver, treating patients with liver-related IEM require specific attention to treatment dosage. A recent case describes a patient with Rotor syndrome (MIM 237450) who developed ovarian cancer.[198] Rotor syndrome is a rare congenital disorder characterized by functional hyperbilirubinemia. The ovarian cancer was treated with Paclitaxel, a drug metabolized in the liver and excreted into the bile. The treating physicians reduced Paclitaxel dosage by 50% based on the diagnosis of Rotor syndrome and the patient experienced neither clinical nor biochemical derangement, and treatment was well tolerated.[198]

In contrast, another paper describes two patients with metastatic colon cancer and Gilbert syndrome (MIM 143500) treated with CPT-11-based chemotherapy who experienced severe toxicity.[199] Patients with Gilbert syndrome have deficient uridine diphosphate glucuronosyl

transferases (UGTs [MIM 191740]), and the drug CPT-11 is hydrolyzed to its active metabolite through conjugation by UGT. Hence, in all cycles, patients showed evidence of severe neutropenia and diarrhea, and treatment of patients with Gilbert syndrome should anticipate this increase in toxicity.

A different example of chemotherapy and toxicity in IEM involves hematological abnormalities such as those seen in GD. Thrombocytopenia is a common finding in patients with GD, resulting from two related mechanisms, decreased platelet production in the bone marrow and splenic sequestration. Other clinical manifestations reflecting the hematological involvement of this disease include anemia, bleeding, and pancytopenia. Cancer treatment of GD patients with chemotherapy has often been discontinued because hematological toxicity due to chemotherapy exacerbated the pancytopenia already present in GD, emphasizing the need for the right regimen.[200]

Together, these cases demonstrate that better understanding of the pathophysiology of the IEM leads to a better choice of anticancer therapy and thus improves the treatment of cancer in these patients.

Summary

Multiple evidences suggest an intimate link between tumorigenesis and disrupted metabolic regulation (Figure 5). By following the Vogelstein model for multistep accumulation of mutation for carcinogenesis,[201] one can understand how an IEM can lead to increased mutagenesis by causing increasing DNA damage (Figure 1). Thus, not surprisingly, long-term follow up of patients with IEM is starting to reveal specific patterns of cancer susceptibility (Table 1).

In IEM, the initiation of carcinogenesis starts from germ line mutations in either the mtDNA or nuclear DNA. This core disruptive event in metabolism predisposes cells to malignant transformation by a variety of putative mechanisms including increased ROS and depletion of the mitochondrial dNTP pools, as well as accumulation of toxic metabolites or a compensatory channeling of metabolites to an alternative pathway. The rarity of IEM disorders and the frequency of cancer development make a cause and effect argument clear. Similarly, the observation that specific IEM tend to develop unique types of cancer further argues against coincidental findings and suggests that the specific metabolic change is involved in the development of the specific cancer. However, as described here, the prevalence of HCC in a variety of IEM also argue that premature cirrhosis and other events might constitute a shared pathophysiologic pathway.

Hence, IEM patients offer an opportunity to understand specific cancer pathogenesis that could aid in developing rationale treatments relevant for these types of cancers when they occur sporadically. Because IEM in general are rare disorders, the quantification of cancer risk in each disorder is difficult. We hope this review will bring increased awareness to the cancer susceptibility in the described IEM and lead to larger collaborative studies to address important clinical questions in this population.

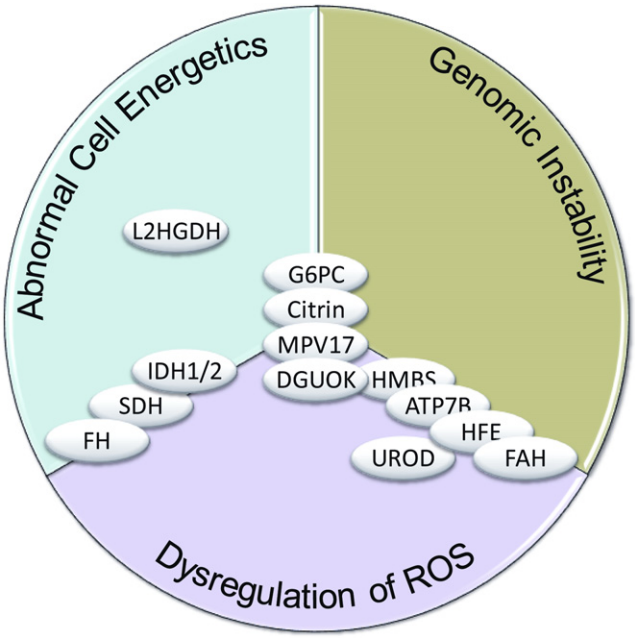

Figure 5. The Summary of Converging Mechanisms in IEM Underlying Tumorigenesis

Abnormal cell energy metabolism encompasses Warburg effect, increased flux through P5P shunt, increased synthesis of fatty acids and triglycerides, and glutamine dependence, which can be seen patients with IEMs. Alterations in the nucleotide pools, mtDNA mutations, and direct mutagenesis by abnormal intermediate metabolites come under the general mechanism of genomic instability. Finally, alterations in the ROS biology can either promote or inhibit cellular growth depending on the intracellular context. Abnormalities in the energy metabolism, genomic instability, and altered ROS biology interact and might amplify each other. Each disorder might have one or more mechanisms involved in tumorigenesis. The net effect of these changes is not necessarily direct tumorigenesis, but rather decreased threshold for tumor transformation.

Acknowledgments

S.E.P. is supported by CA138836-01. A.E. is supported by DK081735-01A1, National Institutes of Health/ National Institute of General Medical Sciences T32 contract grant number GM07526 and by the National Urea Cycle Foundation Research Fellowship. O.A.S. received support through the 2009 National Urea Cycle Foundation Research Fellowship. B.L. is supported in part by the Cancer Prevention Institute of Texas and TR01 GM090310. F.S. is supported by the Gustavus and Louise Pfeiffer Research Foundation Figures were produced by using Servier Medical Art.

Web Resources

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim

References

1. Alexander, F.E., Patheal, S.L., Biondi, A., Brandalise, S., Cabrera, M.E., Chan, L.C., Chen, Z., Cimino, G., Cordoba, J.C., Gu, L.J., et al. (2001). Transplacental chemical exposure

and risk of infant leukemia with MLL gene fusion. Cancer Res. *61*, 2542–2546.
2. Warburg, O. (1956). On the origin of cancer cells. Science *123*, 309–314.
3. Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science *324*, 1029–1033.
4. Luo, J., Manning, B.D., and Cantley, L.C. (2003). Targeting the PI3K-Akt pathway in human cancer: Rationale and promise. Cancer Cell *4*, 257–262.
5. Lanpher, B., Brunetti-Pierri, N., and Lee, B. (2006). Inborn errors of metabolism: The flux from Mendelian to complex diseases. Nat. Rev. Genet. *7*, 449–460.
6. Marsden, D., Larson, C., and Levy, H.L. (2006). Newborn screening for metabolic disorders. J. Pediatr. *148*, 577–584.
7. Zytkovicz, T.H., Fitzgerald, E.F., Marsden, D., Larson, C.A., Shih, V.E., Johnson, D.M., Strauss, A.W., Comeau, A.M., Eaton, R.B., and Grady, G.F. (2001). Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: A two-year summary from the New England Newborn Screening Program. Clin. Chem. *47*, 1945–1955.
8. Chace, D.H., Kalas, T.A., and Naylor, E.W. (2002). The application of tandem mass spectrometry to neonatal screening for inherited disorders of intermediary metabolism. Annu. Rev. Genomics Hum. Genet. *3*, 17–45.
9. Schwartz, I.V., Souza, C.F., and Giugliani, R. (2008). Treatment of inborn errors of metabolism. J. Pediatr. (Rio J.) *84* (4, Suppl), S8–S19.
10. Waisbren, S.E., Albers, S., Amato, S., Ampola, M., Brewster, T.G., Demmer, L., Eaton, R.B., Greenstein, R., Korson, M., Larson, C., et al. (2003). Effect of expanded newborn screening for biochemical genetic disorders on child outcomes and parental stress. JAMA *290*, 2564–2572.
11. Onal, I.K., Turhan, N., Oztas, E., Arhan, M., Akcoren, Z., Oguz, P., Akdogan, M., Onal, E.D., Kacar, S., Kurt, M., and Sasmaz, N. (2009). Hepatocellular carcinoma in an adult patient with type IV glycogen storage disease. Acta Gastroenterol. Belg. *72*, 377–378.
12. de Moor, R.A., Schweizer, J.J., van Hoek, B., Wasser, M., Vink, R., and Maaswinkel-Mooy, P.D. (2000). Hepatocellular carcinoma in glycogen storage disease type IV. Arch. Dis. Child. *82*, 479–480.
13. Burnichon, N., Brière, J.J., Libé, R., Vescovo, L., Rivière, J., Tissier, F., Jouanno, E., Jeunemaitre, X., Bénit, P., Tzagoloff, A., et al. (2010). SDHA is a tumor suppressor gene causing paraganglioma. Hum. Mol. Genet. *19*, 3011–3020.
14. Brusilow, S.W., and Horwich, A.L. (2009). Amino Acids: Urea Cycle Enzymes. In Metabolic and Molecular Bases of Inherited Disease, Eighth Edition, Chapter 8, C. Scriver, et al, eds. (New York: McGraw-Hill). www.ommbid.com
15. Broedbaek, K., Poulsen, H.E., Weimann, A., Kom, G.D., Schwedhelm, E., Nielsen, P., and Böger, R.H. (2009). Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in hereditary hemochromatosis. Free Radic. Biol. Med. *47*, 1230–1233.
16. Kaelin, W.G., Jr. (2009). SDH5 mutations and familial paraganglioma: Somewhere Warburg is smiling. Cancer Cell *16*, 180–182.
17. Litten, J.B., and Tomlinson, G.E. (2008). Liver tumors in children. Oncologist *13*, 812–820.
18. Soeda, J., Yazaki, M., Nakata, T., Miwa, S., Ikeda, S., Hosoda, W., Iijima, M., Kobayashi, K., Saheki, T., Kojiro, M., and Miyagawa, S. (2008). Primary liver carcinoma exhibiting dual hepatocellular-biliary epithelial differentiations associated with citrin deficiency: A case report. J. Clin. Gastroenterol. *42*, 855–860.
19. Marhenke, S., Lamlé, J., Buitrago-Molina, L.E., Cañón, J.M., Geffers, R., Finegold, M., Sporn, M., Yamamoto, M., Manns, M.P., Grompe, M., and Vogel, A. (2008). Activation of nuclear factor E2-related factor 2 in hereditary tyrosinemia type 1 and its role in survival and tumor development. Hepatology *48*, 487–496.
20. Harrison, S.A., and Bacon, B.R. (2005). Relation of hemochromatosis with hepatocellular carcinoma: Epidemiology, natural history, pathophysiology, screening, treatment, and prevention. Med. Clin. North Am. *89*, 391–409.
21. Lithner, F., and Wetterberg, L. (1984). Hepatocellular carcinoma in patients with acute intermittent porphyria. Acta Med. Scand. *215*, 271–274.
22. Savas, N., Canan, O., Ozcay, F., Bilezikci, B., Karakayali, H., Yilmaz, U., and Haberal, M. (2006). Hepatocellular carcinoma in Wilson's disease: A rare association in childhood. Pediatr. Transplant. *10*, 639–643.
23. Xu, R., Mistry, P., McKenna, G., Emre, S., Schiano, T., Bu-Ghanim, M., Levi, G., and Fiel, M.I. (2005). Hepatocellular carcinoma in type 1 Gaucher disease: A case report with review of the literature. Semin. Liver Dis. *25*, 226–229.
24. Franco, L.M., Krishnamurthy, V., Bali, D., Weinstein, D.A., Arn, P., Clary, B., Boney, A., Sullivan, J., Frush, D.P., Chen, Y.T., and Kishnani, P.S. (2005). Hepatocellular carcinoma in glycogen storage disease type Ia: A case series. J. Inherit. Metab. Dis. *28*, 153–162.
25. Demo, E., Frush, D., Gottfried, M., Koepke, J., Boney, A., Bali, D., Chen, Y.T., and Kishnani, P.S. (2007). Glycogen storage disease type III-hepatocellular carcinoma a long-term complication? J. Hepatol. *46*, 492–498.
26. Kok, K.F., Wahab, P.J., Houwen, R.H., Drenth, J.P., de Man, R.A., van Hoek, B., Meijer, J.W., Willekens, F.L., and de Vries, R.A. (2007). Heterozygous alpha-I antitrypsin deficiency as a co-factor in the development of chronic liver disease: A review. Neth. J. Med. *65*, 160–166.
27. Yin, P.H., Lee, H.C., Chau, G.Y., Wu, Y.T., Li, S.H., Lui, W.Y., Wei, Y.H., Liu, T.Y., and Chi, C.W. (2004). Alteration of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma. Br. J. Cancer *90*, 2390–2396.
28. Dragani, T.A. (2010). Risk of HCC: Genetic heterogeneity and complex genetics. J. Hepatol. *52*, 252–257.
29. Di Rocco, M., Calevo, M.G., Taro', M., Melis, D., Allegri, A.E., and Parenti, G. (2008). Hepatocellular adenoma and metabolic balance in patients with type Ia glycogen storage disease. Mol. Genet. Metab. *93*, 398–402.
30. St-Louis, M., and Tanguay, R.M. (1997). Mutations in the fumarylacetoacetate hydrolase gene causing hereditary tyrosinemia type I: Overview. Hum. Mutat. *9*, 291–299.
31. Pitkänen, S.T., Salo, M.K., and Heikinheimo, M. (2000). Hereditary tyrosinaemia type I: From basics to progress in treatment. Ann. Med. *32*, 530–538.
32. Grompe, M. (2001). The pathophysiology and treatment of hereditary tyrosinemia type 1. Semin. Liver Dis. *21*, 563–571.
33. Manabe, S., Sassa, S., and Kappas, A. (1985). Hereditary tyrosinemia. Formation of succinylacetone-amino acid adducts. J. Exp. Med. *162*, 1060–1074.
34. Jorquera, R., and Tanguay, R.M. (1997). The mutagenicity of the tyrosine metabolite, fumarylacetoacetate, is enhanced by

glutathione depletion. Biochem. Biophys. Res. Commun. *232*, 42–48.
35. Weinberg, A.G., Mize, C.E., and Worthen, H.G. (1976). The occurrence of hepatoma in the chronic form of hereditary tyrosinemia. J. Pediatr. *88*, 434–438.
36. van Spronsen, F.J., Thomasse, Y., Smit, G.P., Leonard, J.V., Clayton, P.T., Fidler, V., Berger, R., and Heymans, H.S. (1994). Hereditary tyrosinemia type I: A new clinical classification with difference in prognosis on dietary treatment. Hepatology *20*, 1187–1191.
37. Holme, E., and Lindstedt, S. (2000). Nontransplant treatment of tyrosinemia. Clin. Liver Dis. *4*, 805–814.
38. Feder, J.N., Gnirke, A., Thomas, W., Tsuchihashi, Z., Ruddy, D.A., Basava, A., Dormishian, F., Domingo, R., Jr., Ellis, M.C., Fullan, A., et al. (1996). A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat. Genet. *13*, 399–408.
39. Franchini, M., and Veneri, D. (2005). Recent advances in hereditary hemochromatosis. Ann. Hematol. *84*, 347–352.
40. Kowdley, K.V., Hassanein, T., Kaur, S., Farrell, F.J., Van Thiel, D.H., Keeffe, E.B., Sorrell, M.F., Bacon, B.R., Weber, F.L., Jr., and Tavill, A.S. (1995). Primary liver cancer and survival in patients undergoing liver transplantation for hemochromatosis. Liver Transpl. Surg. *1*, 237–241.
41. Nelson, R.L., Davis, F.G., Persky, V., and Becker, E. (1995). Risk of neoplastic and other diseases among people with heterozygosity for hereditary hemochromatosis. Cancer *76*, 875–879.
42. Gannon, P.O., Medelci, S., Le Page, C., Beaulieu, M., Provencher, D.M., Mes-Masson, A.M., and Santos, M.M. (2010). Impact of hemochromatosis gene (HFE) mutations on epithelial ovarian cancer risk and prognosis. Int. J. Cancer., in press. Published online July 28, 2010. 10.1002/ijc.25577.
43. Osborne, N.J., Gurrin, L.C., Allen, K.J., Constantine, C.C., Delatycki, M.B., McLaren, C.E., Gertig, D.M., Anderson, G.J., Southey, M.C., Olynyk, J.K., et al. (2010). HFE C282Y homozygotes are at increased risk of breast and colorectal cancer. Hepatology *51*, 1311–1318.
44. Kew, M.D. (1990). Pathogenesis of hepatocellular carcinoma in hereditary hemochromatosis: Occurrence in noncirrhotic patients. Hepatology *11*, 1086–1087.
45. Toyokuni, S. (2009). Role of iron in carcinogenesis: Cancer as a ferrotoxic disease. Cancer Sci. *100*, 9–16.
46. Zacharski, L.R., Chow, B.K., Howes, P.S., Shamayeva, G., Baron, J.A., Dalman, R.L., Malenka, D.J., Ozaki, C.K., and Lavori, P.W. (2008). Decreased cancer risk after iron reduction in patients with peripheral arterial disease: Results from a randomized trial. J. Natl. Cancer Inst. *100*, 996–1002.
47. Hardell, L., Bengtsson, N.O., Jonsson, U., Eriksson, S., and Larsson, L.G. (1984). Aetiological aspects on primary liver cancer with special regard to alcohol, organic solvents and acute intermittent porphyria—an epidemiological investigation. Br. J. Cancer *50*, 389–397.
48. Linet, M.S., Gridley, G., Nyrén, O., Mellemkjaer, L., Olsen, J.H., Keehn, S., Adami, H.O., and Fraumeni, J.F., Jr. (1999). Primary liver cancer, other malignancies, and mortality risks following porphyria: A cohort study in Denmark and Sweden. Am. J. Epidemiol. *149*, 1010–1015.
49. Puy, H., Gross, U., Deybach, J.C., Robréau, A.M., Frank, M., Nordmann, Y., and Doss, M. (1998). Exon 1 donor splice site mutations in the porphobilinogen deaminase gene in the non-erythroid variant form of acute intermittent porphyria. Hum. Genet. *103*, 570–575.
50. Herrick, A.L., and McColl, K.E. (2005). Acute intermittent porphyria. Best Pract. Res. Clin. Gastroenterol. *19*, 235–249.
51. Andersson, C., Bjersing, L., and Lithner, F. (1996). The epidemiology of hepatocellular carcinoma in patients with acute intermittent porphyria. J. Intern. Med. *240*, 195–201.
52. Kushner, J.P., Barbuto, A.J., and Lee, G.R. (1976). An inherited enzymatic defect in porphyria cutanea tarda: Decreased uroporphyrinogen decarboxylase activity. J. Clin. Invest. *58*, 1089–1097.
53. Frank, J., and Poblete-Gutiérrez, P. (2010). Porphyria cutanea tarda—when skin meets liver. Best Pract. Res. Clin. Gastroenterol. *24*, 735–745.
54. Bulaj, Z.J., Phillips, J.D., Ajioka, R.S., Franklin, M.R., Griffen, L.M., Guinee, D.J., Edwards, C.Q., and Kushner, J.P. (2000). Hemochromatosis genes and other factors contributing to the pathogenesis of porphyria cutanea tarda. Blood *95*, 1565–1571.
55. Douki, T., Onuki, J., Medeiros, M.H., Bechara, E.J., Cadet, J., and Di Mascio, P. (1998). DNA alkylation by 4,5-dioxovaleric acid, the final oxidation product of 5-aminolevulinic acid. Chem. Res. Toxicol. *11*, 150–157.
56. Palmieri, C., Vigushin, D.M., and Peters, T.J. (2004). Managing malignant disease in patients with porphyria. QJM *97*, 115–126.
57. de Bie, P., Muller, P., Wijmenga, C., and Klomp, L.W. (2007). Molecular pathogenesis of Wilson and Menkes disease: Correlation of mutations with molecular defects and disease phenotypes. J. Med. Genet. *44*, 673–688.
58. Huster, D. (2010). Wilson disease. Best Pract. Res. Clin. Gastroenterol. *24*, 531–539.
59. Walshe, J.M., Waldenström, E., Sams, V., Nordlinder, H., and Westermark, K. (2003). Abdominal malignancies in patients with Wilson's disease. QJM *96*, 657–662.
60. Wilkinson, M.L., Portmann, B., and Williams, R. (1983). Wilson's disease and hepatocellular carcinoma: Possible protective role of copper. Gut *24*, 767–771.
61. Wu, J., Forbes, J.R., Chen, H.S., and Cox, D.W. (1994). The LEC rat has a deletion in the copper transporting ATPase gene homologous to the Wilson disease gene. Nat. Genet. *7*, 541–545.
62. Masuda, R., Yoshida, M.C., Sasaki, M., Dempo, K., and Mori, M. (1988). High susceptibility to hepatocellular carcinoma development in LEC rats with hereditary hepatitis. Jpn. J. Cancer Res. *79*, 828–835.
63. Zhang, Y., Li, M., Yao, Q., and Chen, C. (2009). Roles and mechanisms of copper transporting ATPases in cancer pathogenesis. Med. Sci. Monit. *15*, RA1–RA5.
64. Jmoudiak, M., and Futerman, A.H. (2005). Gaucher disease: Pathological mechanisms and modern management. Br. J. Haematol. *129*, 178–188.
65. Bertram, H.C., Eldibany, M., Padgett, J., and Dragon, L.H. (2003). Splenic lymphoma arising in a patient with Gaucher disease. A case report and review of the literature. Arch. Pathol. Lab. Med. *127*, e242–e245.
66. Böhm, P., Kunz, W., Horny, H.P., and Einsele, H. (2001). Adult Gaucher disease in association with primary malignant bone tumors. Cancer *91*, 457–462.
67. Balreira, A., Lacerda, L., Miranda, C.S., and Arosa, F.A. (2005). Evidence for a link between sphingolipid metabolism and expression of CD1d and MHC-class II: Monocytes from Gaucher disease patients as a model. Br. J. Haematol. *129*, 667–676.

68. Hughes, D.A. (2009). Enzyme, substrate, and myeloma in Gaucher disease. Am. J. Hematol. *84*, 199–201.
69. Compston, J.E. (2002). Bone marrow and bone: A functional unit. J. Endocrinol. *173*, 387–394.
70. de Fost, M., Vom Dahl, S., Weverling, G.J., Brill, N., Brett, S., Häussinger, D., and Hollak, C.E. (2006). Increased incidence of cancer in adult Gaucher disease in Western Europe. Blood Cells Mol. Dis. *36*, 53–58.
71. Taddei, T.H., Kacena, K.A., Yang, M., Yang, R., Malhotra, A., Boxer, M., Aleck, K.A., Rennert, G., Pastores, G.M., and Mistry, P.K. (2009). The underrecognized progressive nature of N370S Gaucher disease and assessment of cancer risk in 403 patients. Am. J. Hematol. *84*, 208–214.
72. Chappell, S., Daly, L., Morgan, K., Guetta Baranes, T., Roca, J., Rabinovich, R., Millar, A., Donnelly, S.C., Keatings, V., MacNee, W., et al. (2006). Cryptic haplotypes of SERPINA1 confer susceptibility to chronic obstructive pulmonary disease. Hum. Mutat. *27*, 103–109.
73. Crystal, R.G. (1990). Alpha 1-antitrypsin deficiency, emphysema, and liver disease. Genetic basis and strategies for therapy. J. Clin. Invest. *85*, 1343–1352.
74. Abboud, R.T., Ford, G.T., and Chapman, K.R.; Standards Committee of the Canadian Thoracic Society. (2001). Alpha1-antitrypsin deficiency: A position statement of the Canadian Thoracic Society. Can. Respir. J. *8*, 81–88.
75. Köhnlein, T., and Welte, T. (2008). Alpha-1 antitrypsin deficiency: Pathogenesis, clinical presentation, diagnosis, and treatment. Am. J. Med. *121*, 3–9.
76. McGlynn, K.A., Rosvold, E.A., Lustbader, E.D., Hu, Y., Clapper, M.L., Zhou, T., Wild, C.P., Xia, X.L., Baffoe-Bonnie, A., Ofori-Adjei, D., et al. (1995). Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B1. Proc. Natl. Acad. Sci. USA *92*, 2384–2387.
77. Perlmutter, D.H. (2002). Liver injury in alpha1-antitrypsin deficiency: An aggregated protein induces mitochondrial injury. J. Clin. Invest. *110*, 1579–1583.
78. Manekeller, S., Sauerbruch, T., Fischer, H.P., Propping, P., and Hirner, A. (2010). Heterozygous alpha-1-antitrypsin deficiency (PiMZ): Risk factor in the development of primary liver carcinoma in non-cirrhotic liver? Z. Gastroenterol. *48*, 1211–1214.
79. Teckman, J.H., An, J.K., Blomenkamp, K., Schmidt, B., and Perlmutter, D. (2004). Mitochondrial autophagy and injury in the liver in alpha 1-antitrypsin deficiency. Am. J. Physiol. Gastrointest. Liver Physiol. *286*, G851–G862.
80. Cassiman, D., Claes, K., Lerut, E., Oyen, R., Joniau, S., Van Damme, B., and Jaeken, J. (2007). Bilateral renal cell carcinoma development in long-term Fabry disease. J. Inherit. Metab. Dis. *30*, 830–831.
81. Blanco, J., Herrero, J., Arias, L.F., Garcia-Miralles, N., Gamez, C., and Barrientos, A. (2005). Renal variant of Anderson-Fabry disease and bilateral renal cell carcinoma. Pathol. Res. Pract. *200*, 857–860.
82. Nance, C.S., Klein, C.J., Banikazemi, M., Dikman, S.H., Phelps, R.G., McArthur, J.C., Rodriguez, M., and Desnick, R.J. (2006). Later-onset Fabry disease: An adult variant presenting with the cramp-fasciculation syndrome. Arch. Neurol. *63*, 453–457.
83. Mehta, A., Beck, M., Eyskens, F., Feliciani, C., Kantola, I., Ramaswami, U., Rolfs, A., Rivera, A., Waldek, S., and Germain, D.P. (2010). Fabry disease: A review of current management strategies. QJM *103*, 641–659.
84. Wang, R.Y., Lelis, A., Mirocha, J., and Wilcox, W.R. (2007). Heterozygous Fabry women are not just carriers, but have a significant burden of disease and impaired quality of life. Genet. Med. *9*, 34–45.
85. Young, C.D., and Anderson, S.M. (2008). Sugar and fat - that's where it's at: Metabolic changes in tumors. Breast Cancer Res. *10*, 202.
86. Barger, J.F., and Plas, D.R. (2010). Balancing biosynthesis and bioenergetics: Metabolic programs in oncogenesis. Endocr. Relat. Cancer *17*, R287–R304.
87. Robb, L., Lyons, I., Li, R., Hartley, L., Köntgen, F., Harvey, R.P., Metcalf, D., and Begley, C.G. (1995). Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. Proc. Natl. Acad. Sci. USA *92*, 7075–7079.
88. Lei, K.J., Shelly, L.L., Pan, C.J., Sidbury, J.B., and Chou, J.Y. (1993). Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a. Science *262*, 580–583.
89. Rake, J.P., Visser, G., Labrune, P., Leonard, J.V., Ullrich, K., and Smit, G.P. (2002). Glycogen storage disease type I: Diagnosis, management, clinical course and outcome. Results of the European Study on Glycogen Storage Disease Type I (ESGSD I). Eur. J. Pediatr. *161* (*Suppl 1*), S20–S34.
90. Chou, J.Y., and Mansfield, B.C. (2008). Mutations in the glucose-6-phosphatase-alpha (G6PC) gene that cause type Ia glycogen storage disease. Hum. Mutat. *29*, 921–930.
91. Shen, J., Bao, Y., Liu, H.M., Lee, P., Leonard, J.V., and Chen, Y.T. (1996). Mutations in exon 3 of the glycogen debranching enzyme gene are associated with glycogen storage disease type III that is differentially expressed in liver and muscle. J. Clin. Invest. *98*, 352–357.
92. Endo, Y., Horinishi, A., Vorgerd, M., Aoyama, Y., Ebara, T., Murase, T., Odawara, M., Podskarbi, T., Shin, Y.S., and Okubo, M. (2006). Molecular analysis of the AGL gene: Heterogeneity of mutations in patients with glycogen storage disease type III from Germany, Canada, Afghanistan, Iran, and Turkey. J. Hum. Genet. *51*, 958–963.
93. Siciliano, M., De Candia, E., Ballarin, S., Vecchio, F.M., Servidei, S., Annese, R., Landolfi, R., and Rossi, L. (2000). Hepatocellular carcinoma complicating liver cirrhosis in type IIIa glycogen storage disease. J. Clin. Gastroenterol. *31*, 80–82.
94. Bruno, C., van Diggelen, O.P., Cassandrini, D., Gimpelev, M., Giuffrè, B., Donati, M.A., Introvini, P., Alegria, A., Assereto, S., Morandi, L., et al. (2004). Clinical and genetic heterogeneity of branching enzyme deficiency (glycogenosis type IV). Neurology *63*, 1053–1058.
95. Ozen, H. (2007). Glycogen storage diseases: New perspectives. World J. Gastroenterol. *13*, 2541–2553.
96. Alshak, N.S., Cocjin, J., Podesta, L., van de Velde, R., Makowka, L., Rosenthal, P., and Geller, S.A. (1994). Hepatocellular adenoma in glycogen storage disease type IV. Arch. Pathol. Lab. Med. *118*, 88–91.
97. Labrune, P. (2002). Glycogen storage disease type I: Indications for liver and/or kidney transplantation. Eur. J. Pediatr. *161* (*Suppl 1*), S53–S55.
98. Lee, P.J. (2002). Glycogen storage disease type I: Pathophysiology of liver adenomas. Eur. J. Pediatr. *161* (*Suppl 1*), S46–S49.
99. Bandsma, R.H., Smit, G.P., and Kuipers, F. (2002). Disturbed lipid metabolism in glycogen storage disease type 1. Eur. J. Pediatr. *161* (*Suppl 1*), S65–S69.
100. Kishnani, P.S., Chuang, T.P., Bali, D., Koeberl, D., Austin, S., Weinstein, D.A., Murphy, E., Chen, Y.T., Boyette, K., Liu,

C.H., et al. (2009). Chromosomal and genetic alterations in human hepatocellular adenomas associated with type Ia glycogen storage disease. Hum. Mol. Genet. *18*, 4781–4790.
101. Bourgeron, T., Rustin, P., Chretien, D., Birch-Machin, M., Bourgeois, M., Viegas-Péquignot, E., Munnich, A., and Rötig, A. (1995). Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. Nat. Genet. *11*, 144–149.
102. Tischler, A.S. (2008). Pheochromocytoma and extra-adrenal paraganglioma: Updates. Arch. Pathol. Lab. Med. *132*, 1272–1284.
103. Gimenez-Roqueplo, A.P. (2006). New advances in the genetics of pheochromocytoma and paraganglioma syndromes. Ann. N. Y. Acad. Sci. *1073*, 112–121.
104. Tomlinson, I.P., Alam, N.A., Rowan, A.J., Barclay, E., Jaeger, E.E., Kelsell, D., Leigh, I., Gorman, P., Lamlum, H., Rahman, S., et al; Multiple Leiomyoma Consortium. (2002). Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat. Genet. *30*, 406–410.
105. Hsiao, H.P., Kirschner, L.S., Bourdeau, I., Keil, M.F., Boikos, S.A., Verma, S., Robinson-White, A.J., Nesterova, M., Lacroix, A., and Stratakis, C.A. (2009). Clinical and genetic heterogeneity, overlap with other tumor syndromes, and atypical glucocorticoid hormone secretion in adrenocorticotropin-independent macronodular adrenal hyperplasia compared with other adrenocortical tumors. J. Clin. Endocrinol. Metab. *94*, 2930–2937.
106. Bayley, J.P., Launonen, V., and Tomlinson, I.P. (2008). The FH mutation database: An online database of fumarate hydratase mutations involved in the MCUL (HLRCC) tumor syndrome and congenital fumarase deficiency. BMC Med. Genet. *9*, 20.
107. Stratakis, C.A., and Carney, J.A. (2009). The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis syndrome): Molecular genetics and clinical implications. J. Intern. Med. *266*, 43–52.
108. Maher, E.R., and Eng, C. (2002). The pressure rises: Update on the genetics of phaeochromocytoma. Hum. Mol. Genet. *11*, 2347–2354.
109. Ricketts, C.J., Forman, J.R., Rattenberry, E., Bradshaw, N., Lalloo, F., Izatt, L., Cole, T.R., Armstrong, R., Kumar, V.K., Morrison, P.J., et al. (2010). Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. Hum. Mutat. *31*, 41–51.
110. Guzy, R.D., Sharma, B., Bell, E., Chandel, N.S., and Schumacker, P.T. (2008). Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. Mol. Cell. Biol. *28*, 718–731.
111. Andersen, K.F., Altaf, R., Krarup-Hansen, A., Kromann-Andersen, B., Horn, T., Christensen, N.J., and Hendel, H.W. (2011). Malignant pheochromocytomas and paragangliomas - the importance of a multidisciplinary approach. Cancer Treat. Rev. *37*, 111–119.
112. Baysal, B.E., Willett-Brozick, J.E., Lawrence, E.C., Drovdlic, C.M., Savul, S.A., McLeod, D.R., Yee, H.A., Brackmann, D.E., Slattery, W.H., 3rd, Myers, E.N., et al. (2002). Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. J. Med. Genet. *39*, 178–183.
113. Pollard, P.J., Brière, J.J., Alam, N.A., Barwell, J., Barclay, E., Wortham, N.C., Hunt, T., Mitchell, M., Olpin, S., Moat, S.J., et al. (2005). Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. Hum. Mol. Genet. *14*, 2231–2239.
114. Linehan, W.M., Srinivasan, R., and Schmidt, L.S. (2010). The genetic basis of kidney cancer: A metabolic disease. Nat Rev Urol *7*, 277–285.
115. Slane, B.G., Aykin-Burns, N., Smith, B.J., Kalen, A.L., Goswami, P.C., Domann, F.E., and Spitz, D.R. (2006). Mutation of succinate dehydrogenase subunit C results in increased O2.-, oxidative stress, and genomic instability. Cancer Res. *66*, 7615–7620.
116. Brandon, M., Baldi, P., and Wallace, D.C. (2006). Mitochondrial mutations in cancer. Oncogene *25*, 4647–4662.
117. Lee, S., Nakamura, E., Yang, H., Wei, W., Linggi, M.S., Sajan, M.P., Farese, R.V., Freeman, R.S., Carter, B.D., Kaelin, W.G., Jr., and Schlisio, S. (2005). Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: Developmental culling and cancer. Cancer Cell *8*, 155–167.
118. Topçu, M., Jobard, F., Halliez, S., Coskun, T., Yalçinkayal, C., Gerceker, F.O., Wanders, R.J., Prud'homme, J.F., Lathrop, M., Ozguc, M., and Fischer, J. (2004). L-2-Hydroxyglutaric aciduria: Identification of a mutant gene C14orf160, localized on chromosome 14q22.1. Hum. Mol. Genet. *13*, 2803–2811.
119. Van Schaftingen, E., Rzem, R., and Veiga-da-Cunha, M. (2009). L: -2-Hydroxyglutaric aciduria, a disorder of metabolite repair. J. Inherit. Metab. Dis. *32*, 135–142.
120. Ozişik, P.A., Akalan, N., Palaoğlu, S., and Topçu, M. (2002). Medulloblastoma in a child with the metabolic disease L-2-hydroxyglutaric aciduria. Pediatr. Neurosurg. *37*, 22–26.
121. Moroni, I., Bugiani, M., D'Incerti, L., Maccagnano, C., Rimoldi, M., Bissola, L., Pollo, B., Finocchiaro, G., and Uziel, G. (2004). L-2-hydroxyglutaric aciduria and brain malignant tumors: A predisposing condition? Neurology *62*, 1882–1884.
122. Rogers, R.E., Deberardinis, R.J., Klesse, L.J., Boriack, R.L., Margraf, L.R., and Rakheja, D. (2010). Wilms tumor in a child with L-2-hydroxyglutaric aciduria. Pediatr. Dev. Pathol. *13*, 408–411.
123. Ward, P.S., Patel, J., Wise, D.R., Abdel-Wahab, O., Bennett, B.D., Coller, H.A., Cross, J.R., Fantin, V.R., Hedvat, C.V., Perl, A.E., et al. (2010). The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell *17*, 225–234.
124. Garber, K. (2010). Oncometabolite? IDH1 discoveries raise possibility of new metabolism targets in brain cancers and leukemia. J. Natl. Cancer Inst. *102*, 926–928.
125. Kloosterhof, N.K., Bralten, L.B., Dubbink, H.J., French, P.J., and van den Bent, M.J. (2011). Isocitrate dehydrogenase-1 mutations: A fundamentally new understanding of diffuse glioma? Lancet Oncol. *12*, 83–91.
126. Gross, S., Cairns, R.A., Minden, M.D., Driggers, E.M., Bittinger, M.A., Jang, H.G., Sasaki, M., Jin, S., Schenkein, D.P., Su, S.M., et al. (2010). Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. J. Exp. Med. *207*, 339–344.
127. Dang, L., Jin, S., and Su, S.M. (2010). IDH mutations in glioma and acute myeloid leukemia. Trends Mol. Med. *16*, 387–397.

128. Smeitink, J. (2010). Metabolism, gliomas, and IDH1. N. Engl. J. Med. *362*, 1144–1145.
129. Dang, L., White, D.W., Gross, S., Bennett, B.D., Bittinger, M.A., Driggers, E.M., Fantin, V.R., Jang, H.G., Jin, S., Keenan, M.C., et al. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature *462*, 739–744.
130. Reitman, Z.J., and Yan, H. (2010). Isocitrate dehydrogenase 1 and 2 mutations in cancer: Alterations at a crossroads of cellular metabolism. J. Natl. Cancer Inst. *102*, 932–941.
131. Zhao, S., Lin, Y., Xu, W., Jiang, W., Zha, Z., Wang, P., Yu, W., Li, Z., Gong, L., Peng, Y., et al. (2009). Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science *324*, 261–265.
132. Fu, Y., Huang, R., Du, J., Yang, R., An, N., and Liang, A. (2010). Glioma-derived mutations in IDH: From mechanism to potential therapy. Biochem. Biophys. Res. Commun. *397*, 127–130.
133. Kobayashi, K., Sinasac, D.S., Iijima, M., Boright, A.P., Begum, L., Lee, J.R., Yasuda, T., Ikeda, S., Hirano, R., Terazono, H., et al. (1999). The gene mutated in adult-onset type II citrullinaemia encodes a putative mitochondrial carrier protein. Nat. Genet. *22*, 159–163.
134. Saheki, T., and Kobayashi, K. (2002). Mitochondrial aspartate glutamate carrier (citrin) deficiency as the cause of adult-onset type II citrullinemia (CTLN2) and idiopathic neonatal hepatitis (NICCD). J. Hum. Genet. *47*, 333–341.
135. Dimmock, D., Maranda, B., Dionisi-Vici, C., Wang, J., Kleppe, S., Fiermonte, G., Bai, R., Hainline, B., Hamosh, A., O'Brien, W.E., et al. (2009). Citrin deficiency, a perplexing global disorder. Mol. Genet. Metab. *96*, 44–49.
136. Hagiwara, N., Sekijima, Y., Takei, Y., Ikeda, S., Kawasaki, S., Kobayashi, K., and Saheki, T. (2003). Hepatocellular carcinoma in a case of adult-onset type II citrullinemia. Intern. Med. *42*, 978–982.
137. Kimura, A., Kage, M., Nagata, I., Mushiake, S., Ohura, T., Tazawa, Y., Maisawa, S., Tomomasa, T., Abukawa, D., Okano, Y., et al. (2010). Histological findings in the livers of patients with neonatal intrahepatic cholestasis caused by citrin deficiency. Hepatol. Res. *40*, 295–303.
138. Nagasaka, H., Okano, Y., Tsukahara, H., Shigematsu, Y., Momoi, T., Yorifuji, J., Miida, T., Ohura, T., Kobayashi, K., Saheki, T., et al. (2009). Sustaining hypercitrullinemia, hypercholesterolemia and augmented oxidative stress in Japanese children with aspartate/glutamate carrier isoform 2-citrin-deficiency even during the silent period. Mol. Genet. Metab. *97*, 21–26.
139. Ito, T., Shiraki, K., Sekoguchi, K., Yamanaka, T., Sugimoto, K., Takase, K., Tameda, Y., and Nakano, T. (2000). Hepatocellular carcinoma associated with adult-type citrullinemia. Dig. Dis. Sci. *45*, 2203–2206.
140. Solaini, G., Sgarbi, G., and Baracca, A. (2010). Oxidative phosphorylation in cancer cells. Biochim. Biophys. Acta, in press. Published online September 26, 2010. 10.1016/j.bbabio.2010.09.003.
141. Kunz, B.A., and Kohalmi, S.E. (1991). Modulation of mutagenesis by deoxyribonucleotide levels. Annu. Rev. Genet. *25*, 339–359.
142. Ke, P.Y., Kuo, Y.Y., Hu, C.M., and Chang, Z.F. (2005). Control of dTTP pool size by anaphase promoting complex/cyclosome is essential for the maintenance of genetic stability. Genes Dev. *19*, 1920–1933.
143. Ralph, S.J., Rodríguez-Enríquez, S., Neuzil, J., Saavedra, E., and Moreno-Sánchez, R. (2010). The causes of cancer revisited: "Mitochondrial malignancy" and ROS-induced oncogenic transformation - why mitochondria are targets for cancer therapy. Mol. Aspects Med. *31*, 145–170.
144. Klaunig, J.E., Kamendulis, L.M., and Hocevar, B.A. (2010). Oxidative stress and oxidative damage in carcinogenesis. Toxicol. Pathol. *38*, 96–109.
145. Dimmock, D.P., Dunn, J.K., Feigenbaum, A., Rupar, A., Horvath, R., Freisinger, P., Mousson de Camaret, B., Wong, L.J., and Scaglia, F. (2008). Abnormal neurological features predict poor survival and should preclude liver transplantation in patients with deoxyguanosine kinase deficiency. Liver Transpl. *14*, 1480–1485.
146. El-Hattab, A.W., Li, F.Y., Schmitt, E., Zhang, S., Craigen, W.J., and Wong, L.J. (2010). MPV17-associated hepatocerebral mitochondrial DNA depletion syndrome: New patients and novel mutations. Mol. Genet. Metab. *99*, 300–308.
147. Karadimas, C.L., Vu, T.H., Holve, S.A., Chronopoulou, P., Quinzii, C., Johnsen, S.D., Kurth, J., Eggers, E., Palenzuela, L., Tanji, K., et al. (2006). Navajo neurohepatopathy is caused by a mutation in the MPV17 gene. Am. J. Hum. Genet. *79*, 544–548.
148. Spinazzola, A., Viscomi, C., Fernandez-Vizarra, E., Carrara, F., D'Adamo, P., Calvo, S., Marsano, R.M., Donnini, C., Weiher, H., Strisciuglio, P., et al. (2006). MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. Nat. Genet. *38*, 570–575.
149. Scheers, I., Bachy, V., Stephenne, X., and Sokal, E.M. (2005). Risk of hepatocellular carcinoma in liver mitochondrial respiratory chain disorders. J. Pediatr. *146*, 414–417.
150. Kunz, B.A. (1988). Mutagenesis and deoxyribonucleotide pool imbalance. Mutat. Res. *200*, 133–147.
151. Kulawiec, M., Safina, A., Desouki, M.M., Still, I., Matsui, S., Bakin, A., and Singh, K.K. (2008). Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. Cancer Biol. Ther. *7*, 1732–1743.
152. Naito, A., Cook, C.C., Mizumachi, T., Wang, M., Xie, C.H., Evans, T.T., Kelly, T., and Higuchi, M. (2008). Progressive tumor features accompany epithelial-mesenchymal transition induced in mitochondrial DNA-depleted cells. Cancer Sci. *99*, 1584–1588.
153. Akman, H.O., Dorado, B., López, L.C., García-Cazorla, A., Vilà, M.R., Tanabe, L.M., Dauer, W.T., Bonilla, E., Tanji, K., and Hirano, M. (2008). Thymidine kinase 2 (H126N) knockin mice show the essential role of balanced deoxynucleotide pools for mitochondrial DNA maintenance. Hum. Mol. Genet. *17*, 2433–2440.
154. López, L.C., Akman, H.O., García-Cazorla, A., Dorado, B., Martí, R., Nishino, I., Tadesse, S., Pizzorno, G., Shungu, D., Bonilla, E., et al. (2009). Unbalanced deoxynucleotide pools cause mitochondrial DNA instability in thymidine phosphorylase-deficient mice. Hum. Mol. Genet. *18*, 714–722.
155. Dallabona, C., Marsano, R.M., Arzuffi, P., Ghezzi, D., Mancini, P., Zeviani, M., Ferrero, I., and Donnini, C. (2010). Sym1, the yeast ortholog of the MPV17 human disease protein, is a stress-induced bioenergetic and morphogenetic mitochondrial modulator. Hum. Mol. Genet. *19*, 1098–1107.
156. Fukuhara, N., Tokiguchi, S., Shirakawa, K., and Tsubaki, T. (1980). Myoclonus epilepsy associated with ragged-red fibres (mitochondrial abnormalities): Disease entity or a syndrome? Light-and electron-microscopic studies of two cases and review of literature. J. Neurol. Sci. *47*, 117–133.

157. Holme, E., Larsson, N.G., Oldfors, A., Tulinius, M., Sahlin, P., and Stenman, G. (1993). Multiple symmetric lipomas with high levels of mtDNA with the tRNA(Lys) A—>G(8344) mutation as the only manifestation of disease in a carrier of myoclonus epilepsy and ragged-red fibers (MERRF) syndrome. Am. J. Hum. Genet. *52*, 551–556.
158. Yoneda, M., Tanno, Y., Horai, S., Ozawa, T., Miyatake, T., and Tsuji, S. (1990). A common mitochondrial DNA mutation in the t-RNA(Lys) of patients with myoclonus epilepsy associated with ragged-red fibers. Biochem. Int. *21*, 789–796.
159. Schoffer, K., and Grant, I. (2006). Multiple lipomas, alcoholism, and neuropathy: Madelung's disease or MERRF? Muscle Nerve *33*, 142–146.
160. Teive, H.A., Munhoz, R.P., Muzzio, J.A., Scola, R.H., Kay, C.K., Raskin, S., Werneck, L.C., and Bruhn, H. (2008). Cerebellar ataxia, myoclonus, cervical lipomas, and MERRF syndrome. Case report. Mov. Disord. *23*, 1191–1192.
161. Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. (2008). Autophagy fights disease through cellular self-digestion. Nature *451*, 1069–1075.
162. Scott, L., Lamb, J., Smith, S., and Wheatley, D.N. (2000). Single amino acid (arginine) deprivation: Rapid and selective death of cultured transformed and malignant cells. Br. J. Cancer *83*, 800–810.
163. Dillon, B.J., Prieto, V.G., Curley, S.A., Ensor, C.M., Holtsberg, F.W., Bomalaski, J.S., and Clark, M.A. (2004). Incidence and distribution of argininosuccinate synthetase deficiency in human cancers: A method for identifying cancers sensitive to arginine deprivation. Cancer *100*, 826–833.
164. Lagarde, S.M., Ver Loren van Themaat, P.E., Moerland, P.D., Gilhuijs-Pederson, L.A., Ten Kate, F.J., Reitsma, P.H., van Kampen, A.H., Zwinderman, A.H., Baas, F., and van Lanschot, J.J. (2008). Analysis of gene expression identifies differentially expressed genes and pathways associated with lymphatic dissemination in patients with adenocarcinoma of the esophagus. Ann. Surg. Oncol. *15*, 3459–3470.
165. Kobayashi, E., Masuda, M., Nakayama, R., Ichikawa, H., Satow, R., Shitashige, M., Honda, K., Yamaguchi, U., Shoji, A., Tochigi, N., et al. (2010). Reduced argininosuccinate synthetase is a predictive biomarker for the development of pulmonary metastasis in patients with osteosarcoma. Mol. Cancer Ther. *9*, 535–544.
166. Tytell, A.A., and Neuman, R.E. (1960). Growth response of stable and primary cell cultures to L-ornithine, L-citrulline, and L-arginine. Exp. Cell Res. *20*, 84–91.
167. Kenny, G.E., and Pollock, M.E. (1963). Mammalian cell cultures contaminated with pleuropneumonia-like organisms. I. Effect of pleuropneumonia-like organisms on growth of established cell strains. J. Infect. Dis. *112*, 7–16.
168. Kraemer, P.M. (1964). Mycoplasma (Pplo) from Covertly Contaminated Tissue Cultures: Differences in Arginine Degradation between Strains. Proc. Soc. Exp. Biol. Med. *117*, 910–918.
169. Schimke, R.T., Berlin, C.M., Sweeney, E.W., and Carroll, W.R. (1966). The generation of energy by the arginine dihydrolase pathway in Mycoplasma hominis 07. J. Biol. Chem. *241*, 2228–2236.
170. Kim, R.H., Coates, J.M., Bowles, T.L., McNerney, G.P., Sutcliffe, J., Jung, J.U., Gandour-Edwards, R., Chuang, F.Y., Bold, R.J., and Kung, H.J. (2009). Arginine deiminase as a novel therapy for prostate cancer induces autophagy and caspase-independent apoptosis. Cancer Res. *69*, 700–708.
171. Shen, L.J., Beloussow, K., and Shen, W.C. (2006). Modulation of arginine metabolic pathways as the potential anti-tumor mechanism of recombinant arginine deiminase. Cancer Lett. *231*, 30–35.
172. Izzo, F., Marra, P., Beneduce, G., Castello, G., Vallone, P., De Rosa, V., Cremona, F., Ensor, C.M., Holtsberg, F.W., Bomalaski, J.S., et al. (2004). Pegylated arginine deiminase treatment of patients with unresectable hepatocellular carcinoma: Results from phase I/II studies. J. Clin. Oncol. *22*, 1815–1822.
173. Ascierto, P.A., Scala, S., Castello, G., Daponte, A., Simeone, E., Ottaiano, A., Beneduce, G., De Rosa, V., Izzo, F., Melucci, M.T., et al. (2005). Pegylated arginine deiminase treatment of patients with metastatic melanoma: Results from phase I and II studies. J. Clin. Oncol. *23*, 7660–7668.
174. Severi, T., Vander Borght, S., Libbrecht, L., VanAelst, L., Nevens, F., Roskams, T., Cassiman, D., Fevery, J., Verslype, C., and van Pelt, J.F. (2007). HBx or HCV core gene expression in HepG2 human liver cells results in a survival benefit against oxidative stress with possible implications for HCC development. Chem. Biol. Interact. *168*, 128–134.
175. Pelicano, H., Carney, D., and Huang, P. (2004). ROS stress in cancer cells and therapeutic implications. Drug Resist. Updat. *7*, 97–110.
176. Gogvadze, V., Orrenius, S., and Zhivotovsky, B. (2009). Mitochondria as targets for chemotherapy. Apoptosis *14*, 624–640.
177. Pastorino, J.G., and Hoek, J.B. (2008). Regulation of hexokinase binding to VDAC. J. Bioenerg. Biomembr. *40*, 171–182.
178. Arora, K.K., and Pedersen, P.L. (1988). Functional significance of mitochondrial bound hexokinase in tumor cell metabolism. Evidence for preferential phosphorylation of glucose by intramitochondrially generated ATP. J. Biol. Chem. *263*, 17422–17428.
179. Wallace, D.C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dawn for evolutionary medicine. Annu. Rev. Genet. *39*, 359–407.
180. Mathupala, S.P., Ko, Y.H., and Pedersen, P.L. (2009). Hexokinase-2 bound to mitochondria: Cancer's stygian link to the "Warburg Effect" and a pivotal target for effective therapy. Semin. Cancer Biol. *19*, 17–24.
181. Di Chiro, G., DeLaPaz, R.L., Brooks, R.A., Sokoloff, L., Kornblith, P.L., Smith, B.H., Patronas, N.J., Kufta, C.V., Kessler, R.M., Johnston, G.S., et al. (1982). Glucose utilization of cerebral gliomas measured by [18F] fluorodeoxyglucose and positron emission tomography. Neurology *32*, 1323–1329.
182. Gottlob, K., Majewski, N., Kennedy, S., Kandel, E., Robey, R.B., and Hay, N. (2001). Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. Genes Dev. *15*, 1406–1418.
183. Cao, X., Bloomston, M., Zhang, T., Frankel, W.L., Jia, G., Wang, B., Hall, N.C., Koch, R.M., Cheng, H., Knopp, M.V., and Sun, D. (2008). Synergistic antipancreatic tumor effect by simultaneously targeting hypoxic cancer cells with HSP90 inhibitor and glycolysis inhibitor. Clin. Cancer Res. *14*, 1831–1839.
184. Zhang, X., Varin, E., Briand, M., Allouche, S., Heutte, N., Schwartz, L., Poulain, L., and Icard, P. (2009). Novel therapy for malignant pleural mesothelioma based on anti-energetic

effect: An experimental study using 3-Bromopyruvate on nude mice. Anticancer Res. *29*, 1443–1448.
185. Petros, J.A., Baumann, A.K., Ruiz-Pesini, E., Amin, M.B., Sun, C.Q., Hall, J., Lim, S., Issa, M.M., Flanders, W.D., Hosseini, S.H., et al. (2005). mtDNA mutations increase tumorigenicity in prostate cancer. Proc. Natl. Acad. Sci. USA *102*, 719–724.
186. Shidara, Y., Yamagata, K., Kanamori, T., Nakano, K., Kwong, J.Q., Manfredi, G., Oda, H., and Ohta, S. (2005). Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. Cancer Res. *65*, 1655–1663.
187. Verschoor, M.L., Wilson, L.A., and Singh, G. (2010). Mechanisms associated with mitochondrial-generated reactive oxygen species in cancer. Can. J. Physiol. Pharmacol. *88*, 204–219.
188. Suzuki, H., Kumagai, T., Goto, A., and Sugiura, T. (1998). Increase in intracellular hydrogen peroxide and upregulation of a nuclear respiratory gene evoked by impairment of mitochondrial electron transfer in human cells. Biochem. Biophys. Res. Commun. *249*, 542–545.
189. Burdon, R.H. (1995). Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic. Biol. Med. *18*, 775–794.
190. Preston, T.J., Muller, W.J., and Singh, G. (2001). Scavenging of extracellular H2O2 by catalase inhibits the proliferation of HER-2/Neu-transformed rat-1 fibroblasts through the induction of a stress response. J. Biol. Chem. *276*, 9558–9564.
191. Guyton, K.Z., Liu, Y., Gorospe, M., Xu, Q., and Holbrook, N.J. (1996). Activation of mitogen-activated protein kinase by H2O2. Role in cell survival following oxidant injury. J. Biol. Chem. *271*, 4138–4142.
192. Connor, K.M., Subbaram, S., Regan, K.J., Nelson, K.K., Mazurkiewicz, J.E., Bartholomew, P.J., Aplin, A.E., Tai, Y.T., Aguirre-Ghiso, J., Flores, S.C., and Melendez, J.A. (2005). Mitochondrial H2O2 regulates the angiogenic phenotype via PTEN oxidation. J. Biol. Chem. *280*, 16916–16924.
193. Shimizu, S., Kageyama, M., Yasuda, M., Sasaki, D., Naito, S., Yamamoto, T., and Kiuchi, Y. (2004). Stimulation of in vitro angiogenesis by nitric oxide through the induction of transcription factor ETS-1. Int. J. Biochem. Cell Biol. *36*, 114–122.
194. Sun, X.Z., Vinci, C., Makmura, L., Han, S., Tran, D., Nguyen, J., Hamann, M., Grazziani, S., Sheppard, S., Gutova, M., et al. (2003). Formation of disulfide bond in p53 correlates with inhibition of DNA binding and tetramerization. Antioxid. Redox Signal. *5*, 655–665.
195. Oltersdorf, T., Elmore, S.W., Shoemaker, A.R., Armstrong, R.C., Augeri, D.J., Belli, B.A., Bruncko, M., Deckwerth, T.L., Dinges, J., Hajduk, P.J., et al. (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature *435*, 677–681.
196. Wang, J.L., Liu, D., Zhang, Z.J., Shan, S., Han, X., Srinivasula, S.M., Croce, C.M., Alnemri, E.S., and Huang, Z. (2000). Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. Proc. Natl. Acad. Sci. USA *97*, 7124–7129.
197. Penta, J.S., Johnson, F.M., Wachsman, J.T., and Copeland, W.C. (2001). Mitochondrial DNA in human malignancy. Mutat. Res. *488*, 119–133.
198. Argon, A., Aydiner, A., Tas, F., Saip, P., and Topuz, E. (2002). Safety of paclitaxel in a patient with ovarian cancer and hyperbilirubinemia due to Rotor's syndrome. Gynecol. Oncol. *85*, 362–364.
199. Wasserman, E., Myara, A., Lokiec, F., Goldwasser, F., Trivin, F., Mahjoubi, M., Misset, J.L., and Cvitkovic, E. (1997). Severe CPT-11 toxicity in patients with Gilbert's syndrome: Two case reports. Ann. Oncol. *8*, 1049–1051.
200. Leone, J.P., and Dudek, A.Z. (2008). Enzyme replacement therapy for Gaucher's disease in patient treated for non-small cell lung cancer. Anticancer Res. *28* (*6B*), 3937–3939.
201. Vogelstein, B., and Kinzler, K.W. (1993). The multistep nature of cancer. Trends Genet. *9*, 138–141.